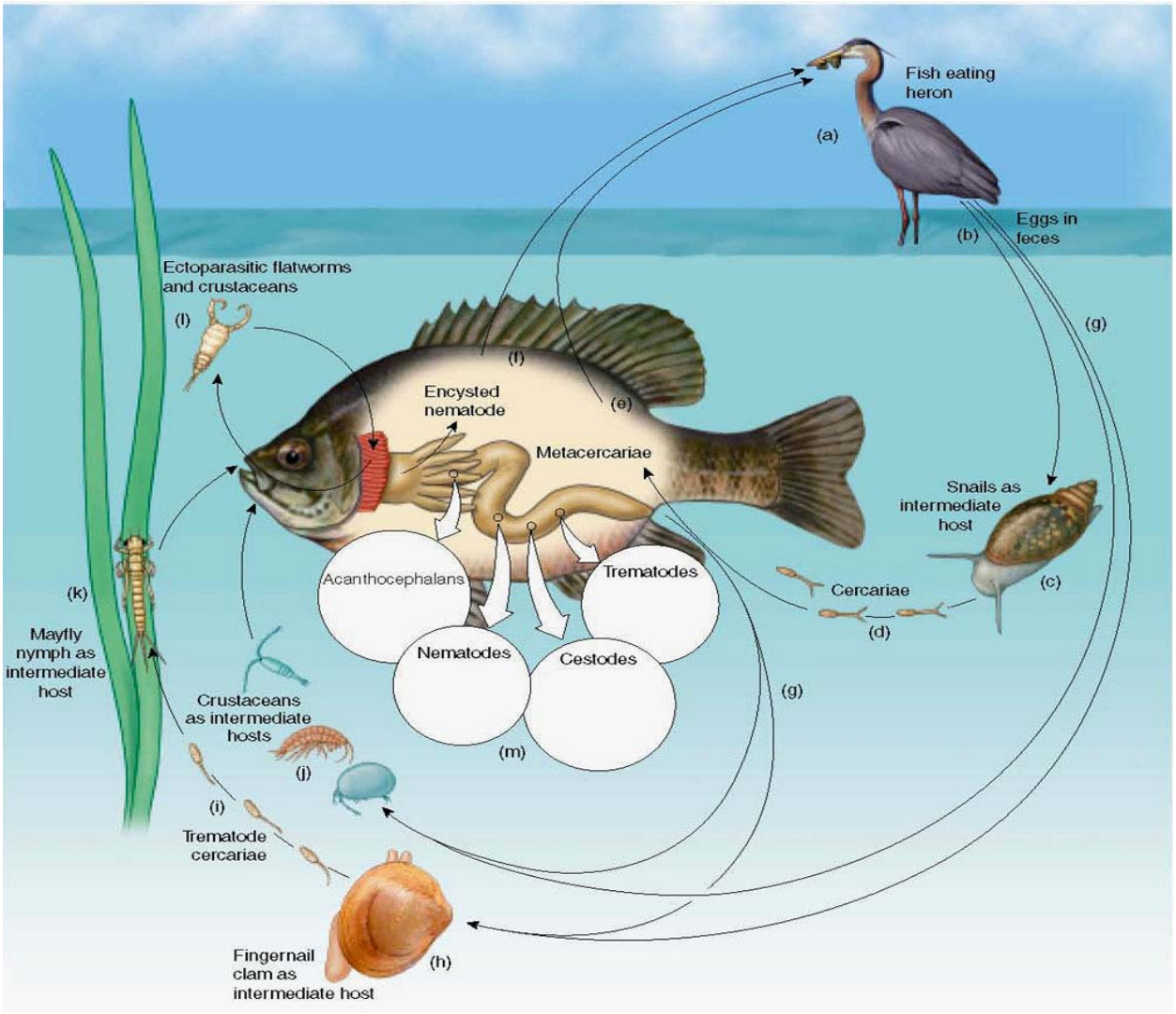
FIELD PARASITOLOGY



Summers
Fifteenth Edition
John Janovy, Jr.

Cedar Point Biological Station

Field Parasitology

Biol Sci 487/887

Summers

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John Janovy, Jr.
Fifteenth Edition
2008

(Cover drawing by Bill Ober for FOUNDATIONS OF PARASITOLOGY, 8th Edition)

FIELD PARASITOLOGY LAB MANUAL
Cedar Point Biological Station
John Janovy, Jr.
(Note: Certain illustrations are missing from the online *.pdf version)

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1. Letter to Students

Welcome to Field Parasitology. I hope your summer will be productive and educational; I know it will be busy. This manual has been put together in order to provide you with the basic information and "tools of the parasitologist" that you will need to complete the course and accomplish the course objectives. There is not much, in this book, in the way of "factual material" that you must learn in order to become a parasitologist. There are, however, many techniques that you will be asked to apply to parasitological problems in the next five weeks. Most of these techniques are not unique to the field of parasitology. Their application to parasitological problems, on the other hand, may present you with some challenges you have not experienced in other courses. You will ultimately be asked to "think parasitologically," and that is one of my major goals for the summer.

Field Parasitology is a field course; it is not a book course. Your time at CPBS will be spent most productively if you concentrate your efforts on original field observations, their interpretation, and their analysis. I find routinely that the most successful students are those who make their own observations and their own interpretations of what they see, not spending too much time on library resources except for help with identification and techniques. Of course there will be "right" and "wrong" answers to the questions you are asked, but most of the time "right" and "wrong" are matters of identification and technique rather than interpretation. Do not be afraid to be creative and original within the context of your basic understanding of biology. Give me the opportunity to do my best job of teaching. That happens when I have a lot of originality to work with, when I am able to decide that the experience is worth more than the results, and when I am able to suggest avenues for exploration from the many choices a student gives me. Your grade will not suffer much from making a "wrong choice" about what to study in the field. It will suffer from not pursuing that choice to the utmost of your ability.

This course requires a fair amount of physical labor. It requires that you actually do things rather than think about them, that you actually make some observations prior to discussing them. In the past, students who have had problems with field work are those who have been reluctant to actually do the physical labor required of the class. Collection, specimen preparation, field notes, and research projects, are all physical acts first, although some of them also involve a touch of artistry. The three-week session goes by very quickly. If you choose a research project during the first week, then also schedule a time that you will, without fail, do the physical labor required of that project. There will be a surprising amount of time near the end of the session for data analysis and writing. But without the data, the observations, you will have nothing to analyze. The observations come from the field.

Parasitology differs from many other areas in that one cannot really deal only with the parasite. Furthermore, most species of animals are parasitized by something. One animal parasitized by another means you must learn two scientific names instead of one. In order to understand the relationship of parasite to host, you may have to study the ecology, behavior, or other aspects of natural history, of both the parasite and the host. Thus the amount of information you must process is doubled, compared to what would be the case if you were studying only the host. If the parasite is one that uses several intermediate hosts, then those additional hosts must also be learned and the manner in which successive hosts interact must be

considered. Usually successive hosts are not taxonomically related. Thus the study of one "subject," parasitology, multiplies the number of kinds of organisms, as well as the number of aspects of their lives, that must be examined. For this reason, the information of parasitology often seems to come in random and unrelated torrents, particularly when that information is in the form of original field observations. Don't be dismayed by all these apparent problems, especially at first. Just try your best to solve them, and be patient with yourself.

The short three-week session places some requirements on the instructor. The foremost of these is the requirement to be as efficient as possible, not wasting any opportunity to make an observation. Furthermore, biological conditions will not always allow an instructor to "schedule" a class exercise. For all these reasons I tend to "take it as it comes" when we go to the field. I will not pass up the chance to point out an organism, or phenomenon, because the opportunity may not exist the next hour, day, or week. Class days in Field Parasitology, then, tend to start out with some objective, usually the collection and analysis of data to illustrate some general parasitological principle. We usually accomplish that objective, but along the way we may also accomplish some others, and your notes at the end of the day might cover a multitude of things, only part of which pertain to the "scheduled" exercise. I think that if you know about this approach beforehand, and understand the reasons why we must use it, then things will go more smoothly.

Finally, the study of parasitology requires some killing. I sympathize with those who resist the killing of wild things. For this reason I have spent many years developing a set of class exercises in which (1) killing of vertebrate animals, with the exception of small fish, is kept to a minimum, and (2) large amounts of data can be collected quickly using species whose biology is most appropriate to illustrate some principle and, I hope, using species which tend to not elicit an emotional response from the average human. It will seem like we do a lot of work on small fish, insects, and snails. The use of such material simply means that if I have a choice of several systems to use to illustrate a principle, I'll pick the one that provides the most data quickly with the least emotional response. Along those same lines, there is a section in the "Class Policies" chapter that deals with the treatment of animals in the lab. Please read that section carefully.

Thanks for enrolling in Field Parasitology. Let's have another exciting summer!

John Janovy, Jr.
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2. How to envision this course:

There are a number of ways to envision the Field Parasitology experience; a few of them are:

(1) As a course in humility and patience

One of the first lessons we learn is that not all plans involving natural materials are easily carried to completion. Thus we are likely to have some class days that simply are not successful for a variety of reasons. My hope is that you will be patient with me and with yourselves when this happens, and take the experience as an authentic lesson in biology, as opposed to the often contrived biology lessons you get in city campus labs. You will also discover that joint efforts, involving a number of people from different backgrounds working toward a common goal always (*always*) take longer to complete than you think they should. Again, patience helps when class days get very long.

(2) As a course in public health

For those of you with interests in the health professions, Field Parasitology probably comes as close as you will come, unless you enroll in a public health program somewhere, to a course in epidemiology, disease distribution, infection rates as influenced by "social" factors, etc. The analytical tools we use in this course are very similar, and in some cases identical, to those used by professional epidemiologists. Indeed, some of the field exercises are very good mimics of tropical medicine research and epidemiological studies.

(3) As a course in microecology

We routinely analyze numbers, distributions, and population structures of parasites that occupy small animals such as insects, crustaceans, and fish. These hosts represent small, patchy, and ephemeral habitats that are occupied by even smaller organisms, their parasites. Thus we tend to use general ecological principles and techniques, and apply them at a microscopic scale.

(4) As a course in biodiversity

By definition, a study of parasitism involves a study of both the host and the parasite, thus two species, and their respective biologies, contribute to the relationship. The widespread (taxonomic) distribution of parasitism means that in five weeks a student can encounter a very large number of species from several phyla.

(5) As a course in pathology and diagnosis

Again, for those with interests in the health professions, Field Parasitology can be thought of as a continuing effort to discover "who" is infected with what and what the effects of that infection might be.

(6) As a course in invertebrate zoology

Many of the hosts we study, and all of the parasites, are invertebrates. You will constantly be asked to lean anatomy, taxonomy, identification, natural history, and ecology of invertebrates. The anatomy in particular may prove to be a challenge for some of you (as well as your instructor).

(7) As a course in the use of the microscope

Field Parasitology will constantly test your microscope skills. It is to your advantage to develop your instrumentation skills, and to work hard at learning to use this most common study device (the microscope). Development of a sense of how to use this instrument will pay off many times during your career.

(8) As a course in teaching

Field Parasitology is designed to illustrate general principles through use of short field exercises. The choice of biological materials is critical to the success of this endeavor. For those of you destined for the teaching profession, this course should help you learn to design studies that rely on easily available biological materials and integrate field work, identification, hypothesis testing, and data analysis.

(9) As a course in learning to deal with complexity

Parasite life cycles, communities, and invertebrate anatomy can all seem highly complex at first, mainly because the animals we encounter are often exotic and small. I try to help students get through their initial shock by (1) repeating certain experiences until these experiences become familiar ones, and (2) asking that you try, early on, the tasks that seem most difficult and unfamiliar.

(10) As a course in learning to generalize

The widespread distribution of parasitism means that you will see the same general phenomena manifested in several different animal groups that at size and numerical scales that vary over an order of magnitude or more. My hope is that you will learn to recognize general phenomena regardless of the scale and circumstance under which they are manifested.

3. Class Policies

(1) Classes meet five days a week; they usually run from 8:00 in the morning to some time in the evening. A typical day consists of an hour or two of lecture and discussion, the remainder of the day in the field, early evening dissecting, identifying, analyzing data, etc., eventually a discussion of the exercise results, sometimes with individual presentations, and finally, cleanup.

(2) There is a set of drawers in the downstairs lab. Please pick one of these and use it however you wish during the session. These drawers can be locked if you choose.

(3) Certain areas of the various CPBS buildings have been designated as research areas. You are welcome to come in my research lab and interrupt me at any time, but other researchers in the same room or building should be respected.

(4) I am available for help about any time.

(5) There are computers available in the library. This course requires that you master a spreadsheet like Excel and one of the statistical packages available for use in class exercises and projects. In the past, I have found that the quickest way to learn to use these packages is simply to use them; in the simpler one (FS or FS2) the commands and results are very self explanatory but the programs are DOS-based and some students resist using such software. In others, you may have to consult manuals, ask for help, and spend some extra time exploring and learning on your own.

(6) At the beginning of the session, you will be assigned certain items of field gear. It is your responsibility to make sure your assigned item is taken to the field, returned to the station, stored properly, and repaired if necessary.

(7) You are expected to know how to use the microscopes. You are also expected to exercise care in their use, keep them clean, and carry out any routine maintenance on them that you can. If there is a problem with a microscope, call it to my attention.

(8) Animal policy: This item is critical to the smooth operation of this course!

a. Do not bring animals into this lab and allow them to languish and die. If you bring animals into the lab, either kill them and dissect them for parasites the same day or let them go.

b. It is possible that your research project may require the keeping of animals for various lengths of time. Please make sure they are properly cared for until the time you are finished with them.

c. Items "a" and "b" above pertain to ALL animals, including invertebrates.

d. It is generally a violation of the law, including federal law in some cases, to kill wild vertebrate animals. My collecting permit covers the species we use in class. Expect to obey the law in your collection of parasite material for this class.

e. Animal remains will be buried nightly in the designated burial ground. There are shovels in the garage. Please bury remains deeply enough so that they won't be dug up by scavengers. It is not the TA's responsibility to actually do this; it is the TA's responsibility to see that it gets done. You will be expected to volunteer for burial detail.

(9) Cleanup: You are expected to clean up the lab each night, or after independent use, so that it is ready for the next class. You are free to use the lab any time.

(l0) Vans: The vans may be used for student projects, and generally for any legitimate educational use associated with the program, however, only those employees whose names are on the annual travel authorization and who have passed UN-L's motor vehicle course may drive the vans. This list includes faculty, teaching assistants, other non-student staff, and occasionally visiting researchers. Vans are to be cleaned out after each day's use.

(11) Security: Please keep an eye out for the safety of cameras, binoculars, etc., your own as well as others. CPBS has never had a security problem and none is expected.

(12) Safety: So far the major problems have been poison ivy, insect stings, flies in the eyes, barbed wire scratches, branches flipping back in people's faces, fingers slammed in van doors, and burns from beach fires. Please look out for your own personal safety in and out of class. **See also the separate chapter on safety issues (chapter 18).**

(13) Personal belongings: Things get very hectic at the end of the session. Please take full responsibility for your own equipment, especially such things as dissecting tools, cameras, calculators, etc. Items left at the end of the sessions usually become CPBS property.

4. Course Requirements

The teaching methods and requirements of Field Parasitology are based on a number of assumptions, the first of these being that there is no way to make an individual a full- fledged parasitologist in three weeks (or three years). The second assumption is that it is quite possible to expose a person to all the tasks of a parasitologist in three weeks. The third assumption is that through proper choice of material, it is possible to incorporate a good deal of quantitative biology, as well as conceptual thinking, into a parasitology course. The exercises that have been developed are intended to be consistent with the above assumptions. Such exercises need to (1) illustrate principles, (2) involve large amounts of data which can be analyzed easily, (3) involve the entire class, (4) be quantitative whenever possible, (5) stimulate far-ranging class discussions and questions, and (6) be accomplished in a single day of about 200 person hours.

However, there are some parasitologist tasks which simply cannot be fitted into a single day because they demand too much individual work. Into this category fall independent research, specimen preparation and curation, preparation of formal oral presentations, and writing a scientific paper. Therefore you will be asked to begin these tasks early in the session and to work at them regularly during the course, usually on your own time.

In general, the course requirements include the following:

(1) Attendance in class and on field trips.

(2) Background lecture information, concepts, facts, etc., much of which is given on field trips, or during class discussions at the end of the day. Some of this material is included in this manual.

(3) A specific set of quantitative tools, i.e. equations and computer programs, which are used in class exercises and in research projects. These tools are discussed in Chapter 11 of this book; a set of PC programs to help you apply these tools, will be available to you.

(4) A set of daily written assignments, e.g. a set of questions or hypotheses based on a previous day's work.

(5) A daily quiz of some kind; in recent years, these quizzes have been practical ones using videotaped material from the previous day's work.

(6) An independent research project *of the student's choosing*, usually done with a partner.

(7) A written report on the independent research project.

(8) An oral presentation on the independent research project, given at the end of the session.

(9) Depending on the year, I may also require a slide collection emphasizing techniques for preparation of a number of different types of parasites, from protozoa to larger helminths and arthropods. If there is not collection required, then we will make some other arrangements for you to see the methods used for specimen preparation and identification.

Obviously this list is a pretty long one, especially considering the fact that you only have three weeks to accomplish it. In several years of teaching Field Parasitology, however, no student has failed to do the overwhelming majority of these requirements in fine fashion. The major failing in recent years has been the artistic quality of permanent slides. In deference to the length of the requirement list, I am pretty tolerant of mistakes in detailed taxonomy, and of other kinds of failures that any experienced biologist knows are unavoidable, but I tend to be fairly intolerant of failure to seriously attempt all the requirements.

Quantitative tools:

Students should expect to be asked to master, to the point of being able to use them in everyday class work and project research, the following concepts and the methods of applying them to field data (see also Chapter 11):

(1) Prevalence, abundance, intensity;

(2) Parasite population distributions among host populations;

(3) The concepts of infrapopulations, component populations, suprapopulations, infracommunities, supracommunities;

(4) Species diversity;

(5) Species density;

(6) Niche breadth;

(7) Niche overlap;

(8) Spatial distributions;

(9) All of the above elaborated demographically, geographically, and by host species.

Data to illustrate the above items are collected, analyzed by the class as a whole, and interpretations made insofar as possible within a parasitological context. You generally have a *lot* of help with the quantitative portions of the course. In fact, we usually end up doing most of the above as regular parts of the class exercises.

Data sheets:

For certain exercises and for most if not all individual projects, you will either design or be provided with data sheets that will help you organize observations, make sure they are consistent from student to student, and to facilitate analysis at the end.

Equipment:

You will need the following, provided at your own expense:

(1) This manual.

(2) A set of dissecting equipment. CPBS has ordered good dissecting equipment that you can purchase.

(3) Diskettes or flash drives for personal computers. I recommend double sided double or high density. Cedar Point usually has a supply of diskettes and blank CDs that you can buy. Computer games are much discouraged, mainly because of the maintenance problems they generate with the common equipment.

(4) Something you can take to the field to take notes on, e.g. a stenographer's pad.

(5) Some kind of a notebook to keep your research project data in.

You are also free to bring anything you want to in terms of your own books, cameras, binoculars, etc. Guns are not necessary and their possession is a violation of university policy. A back pack or field bag is very useful.

So much for the requirements, now for the expectations. I expect students to concentrate as much as possible on original field observations. I expect that if a student is inclined to be creative, scientifically or otherwise, that the student then use that creativity to the utmost. I expect you to make as much use as humanly possible of the unique field opportunities at CPBS, leaving the intense book study for places like Lincoln, where anybody can study books any time. I expect that you will not be afraid to ask questions. I expect that you will have respect for the station library, to use the library materials to supplement, not replace, your field observations. I expect you to try everything you ever wanted to try intellectually back on city campus but were afraid to try because of your grades. And, I expect you to share your knowledge, understanding, and insight with your fellow students during both formal and informal discussions.

Grading policy:

Grading policy will vary depending on the summer, but generally grades are based not only on exams or quizzes, but also on specific performance goals. *You will be given a sheet that indicates the specific graded items and their relative contribution to your grade*. The independent research project is usually worth 35% of the grade, with points being awarded for early choices of projects and early observations, number and kinds of quantitative tools used, quality of oral presentation, and format and quality of written report. The collection when required must include a minimum number of slides of designated kinds of parasites, and has been worth 25% of the grade. I grade each slide in terms of when it is completed, collection data submitted with it, and artistic quality. I usually select the best collection and grade everyone else according to that standard, which generally reflects the most an average student can do with the material and chemicals available for a particular year. If a collection is not required, then I will adjust some other requirements accordingly. Daily written assignments and/or quizzes account for up to 30% of your grade. I reserve the right to award the remaining 10% on a subjective evaluation of your class participation, taking into account effort, attention paid to the exercises,

care of equipment, animals, computers and library materials, and willingness to lead class discussions.

As you can tell from the above, the graded activities are mostly contract work; i.e., if you do the work satisfactorily, you will get full or almost full credit. I adopted this approach to grading many years ago, mainly as a result of watching students progress, or not progress, toward achieving the course goals. It quickly became obvious that if you do certain tasks seriously, you cannot help but come away from the experience educated (cf. some of the paper assignments in my city campus courses). The word "tasks," however, can be somewhat loaded! As a consequence of this pedagogical approach, grades in Field Parasitology generally have been reasonably high, and those who have had trouble with the course have been ones who spent too much time in the library and not enough time in the field and lab, have procrastinated on their project, have not sought help early on use of the quantitative tools, and have been afraid to make their collection.

5. The Concept of a Parasite

Parasites are organisms that live in and on other organisms, in a relationship which is an obligate one for the parasite. The relationship is a intimate one, biochemically and physiologically, and is manifested at the individual level. Individual parasites live in and on individual hosts. Parasites thus occupy an environment which consists of another living organism.

Parasitism is perhaps the most common way of life among animals. All species of "free living" animals which have been studied seriously have been shown to harbor at least one species of parasite. Thus the "free living" animals of the world represent a rich supply of habitats which, of course, are living habitats. The fact that hosts are alive means they provide a regulated supply of carbohydrates, amino acids, lipids, and nucleic acid precursors. Furthermore, living environments are osmotically, and often thermally, regulated. Thus compared to most abiotic environments, the components, and their concentrations, of hosts are relatively regulated, thus predictable. Parasites typically cannot live without their host, a characteristic that can be interpreted as an adaptation to the homeostatic mechanisms of their hosts. Host species also live within their own ecological niches, and the fact that these are somewhat defined suggests that parasites' chances of encountering a host can, theoretically, be optimized through evolution. In summary, one could say that living organisms are particularly attractive environments because organisms that can colonize them are relieved of the biochemical and physiological burdens of regulation. Perhaps such attractiveness is the basis for the pervasive presence of parasites.

All parasites possess mechanisms of transmission. It is critical for a parasite species that individual parasites find hosts. Routinely we refer to infective stages of parasite life cycles. These stages are usually those which also exhibit adaptations for transmission. One of your tasks will be to learn to recognize such stages for what they are. If you are ever in a situation in which you would like to control an epidemic, reduce the prevalence of an infection, etc., the transmission (infective) stage will be one of your prime targets. On the other hand, often the act of transmission involves a host behavioral component. Learn to recognize host behaviors that enhance transmission of animal parasites and you will more easily recognize human behavioral factors which contribute to disease situations.

Parasites often have complex life cycles and students sometimes ask "why?" Questions that begin with "why" are ultimate, rather than proximal, questions and biologists thus usually answer them with explanations of evolutionary history. For example, once a parasite colonized a particular host, its presence in that host may have greatly increased the probability of encountering another potential host species that feeds on the first, and so on. Some feel that such an explanation can account for complex life cycles.

Complex life cycles should also be considered to involve discrete sequential steps in embryological development. Trematode life cycles probably best illustrate this idea. Structures are present in some stages but are not present in other stages (cilia in miracidia, rudimentary digestive tract in redia, tails in cercariae). This observation should be interpreted to mean that

different portions of the genome are being expressed at the different life cycle stages. In this respect, the trematode (or any other parasite with a complex life cycle) is no different from other organisms. However, the parasite is likely to differ from other organisms in that each discrete sub-genome is also expressed in a discrete environment. Thus the transmission act, completed successfully, is the event that triggers expression of the next stage's genes.

Discrete stages of genetic expression, carried out in discrete environments, sets the stage for independent evolution of life cycle stages. Thus a stage may evolve structures and functions unique to that stage, although of course the genes for those structures and functions are a part of the species' genome. Such characters specific to a life cycle stage should contribute positively to the species' survival or reproductive potential, regardless of the stage in which reproduction occurs. Adult parasite colonization of various hosts might well force differences in the evolutionary tracks of immature life cycle stages. Thus cercariae could come to differ among themselves. Such independent evolution of life cycle stages might also account for such things as structural differences between larvae of different mosquito species.

On the other hand, you may find instances in which parasite attributes might be of "historical" origin rather than recent adaptations. For example, the general form of hamuli (large hooks on the haptor of Monogenea) could be shared by a large number and diversity of monogeneans, while the slight variations in proportionate sizes of the hook roots, etc., might be considered characteristics which have evolved as in conjunction with speciation. The major question that evolutionary biologists working with parasites have to answer is: are the observed parasite traits a product of fairly recent adaptations, or are they really constraints, of historical origin, upon the evolution of this group?

Speculations about parasite evolution, for example as given in the above paragraphs, are a part of almost every parasitologist's conversation. Therefore, as part of this course, you will be expected to participate in discussions of evolution, to speculate on the origin of structures and relationships that you observe, and to look for evidence to support (admittedly incomplete) answers to ultimate questions.

Many species of hosts have been colonized by more than one species of parasite. The group of parasite species in a single host is an **assemblage** or a **community**, the difference in these terms being one of implied interactions or physiological relationships between the parasite species. The term assemblage implies little or none, the term community implies some, interaction. In general, there is usually not much obvious evidence for interactions between parasites in individual hosts, although one suspects such interactions must occur, no matter how subtle they may be. Personally I tend to use "assemblage" more than "community," especially in casual conversation, but the latter term is probably most commonly used by other parasitologists. Some host species seem especially receptive to parasites, thus tend to have relatively rich parasite communities. One can usually find in the literature a list of parasite species reported for a host species. This list is one that has accumulated over the years through study of the host by various parasitologists in different areas of the host species' geographic range. You should not expect each host individual to possess all the species of parasites that have been reported for the host species. Likewise, you should not expect a sample of the host population to contain all the species of parasites reported, or even found in the local population. Instead, the reported parasite

list should be considered the maximum theoretical assemblage or community of parasites that could be supported by a host individual or a population. Routinely host species we deal with in this course have an assemblage of parasites, but just as routinely, some hosts support a richer, more diverse, fauna than others. One of the most nagging questions you will have is why hosts differ in the richness of their parasite fauna. A multitude of explanations exist. Thus one of your major tasks will be to discover, and decipher, the biological circumstances that dictate the structure of the parasite assemblage of any one host species.

For pedagogical reasons, we often study organisms that are not necessarily considered parasites, in the sense that whatever harm they may be causing the host is very difficult to observe. Good examples of such organisms might be the ectocommensal ciliates on micro-crustaceans. Quite frankly, it is sometimes very difficult to see "harm" caused by organisms commonly considered "parasites," good examples being some, if not most, tapeworms. Indeed, in the majority of cases, you might have to do years of closely controlled experimental work in order to assess the "harm" caused by parasites, and especially relative "harm" in nature, given the long list of other factors that can have a negative impact on the life of some animal (predation, bad weather, bad luck). So regardless of whether our parasites are harmful to their hosts, I tend to select course materials that are quickly and effectively instructional, and ones that students can actually use to obtain original observations within the time allotted to us.

6. A Parasitological Primer: the ten general rules of parasitism and parasitology

Here are some generalities about parasitism that probably should be kept in mind when assessing the contributions of various people working with host-parasite systems, especially when that work involves parasite population biology, host-parasite co-evolution, and epidemiology or epizootiology:

(1) The first and perhaps most important rule to remember is that the word "parasite" can, and in the primary literature does, refer to an enormous array of organisms ranging from viruses, bacteria, and fungi, to at least some members of all animal phyla (including vertebrates), and numerous plant species. Thus, to borrow a literary device from G. Stein: *a parasite is not a parasite is not a parasite is not a parasite* . . . The more conservative parasitologists would likely recognize at least a dozen or so different types of parasitic relationships; the most liberal parasitologists would probably claim that each host species + parasite species combination is unique in some important way, thus eco-evo-devo work on one system is probably not very applicable, conceptually, to other combinations. A minimal amount of work with any common system in nature, e.g. freshwater fish or amphibians and their parasite communities, shows that even when a single host species is involved, the population-level interactions between that host species and its several parasite species are likely to be parasite species-specific (see Fig. 1). The scientific names of the species involved, both hosts and parasites, are therefore exceedingly important, indeed integral, components of any study because these names are links to our understanding, or lack thereof, of life cycles, developmental requirements, transmission mechanisms, and the like. That is, these names are the search terms for use in gaining access to the primary literature.

(2) The second aspect of parasitism that is critical to an understanding of the phenomenon is host specificity. Thus a second general rule could read: some parasite species (the "specialists") are highly restricted in the kinds of hosts they will infect, whereas others (the "generalists") may be quite unrestricted, although virtually no parasites are universally infective. The extent of generalist and specialist properties may vary at the species level, with different species of a parasite genus exhibiting different levels of host specificity, even at different life cycle stages. One might ask, for example, whether a generalist parasite can indeed drive an evolutionary response from one of its several hosts? Or, perhaps a better question might be: What are the conditions under which a parasite species that routinely infects a dozen host species in nature would drive evolutionary change in only one of those host species?

(3) The third general rule of parasitism is that within any one generation, only a small fraction of genetic diversity among the parasites is displayed against a relatively small fraction of host genetic diversity. This rule is illustrated in Figure 2. The assumption that parasitism can drive evolutionary change also requires an assumption that whatever portion of the parasite's genetic diversity is displayed against a host's genetic diversity in nature is similar from generation to generation, although in nature, pure chance and often highly variable ecological conditions actually determine which fraction of the parasite's genetic repertoire actually survives, and which fraction of the host species' genetic repertoire actually

encounters the parasite survivors. In order to actually show that parasites are driving evolution in any species-to-species relationship, the following conditions must be met:

a. Genetic makeup of an infected host dictates course of infection to the extent of reducing host fitness.

b. The parasite is host specific and is either not affected by host defenses or is genetically variable enough to overcome these defenses in the short term.

c. Succeeding generations of hosts having increasing frequencies of a resistant genotype or decreasing frequencies of a susceptible genotype.

d. The parasite is at or near the top of the list of risk factors for the host; i.e., the parasite cannot be an inconsequential pest.

e. The parasite has to exert its fitness effects on hosts of, or prior to, reproductive age.

f. The ecological arena in which host and parasite encounter one another must be stable enough so that abiotic factors do not override biotic ones in determination of host fitness.

g. Other, co-infecting, parasite species must be shown to have no effects on host fitness.

(4) A fourth general rule is that symbiosis is the most common way of life on Earth and that every organism that has been studied seriously has been found to be occupied, at the species level, by at least one, and typically several, other, unrelated, species, i.e., the symbionts. Thus whatever one person means by the word "parasite," that meaning is likely to fall somewhere on a very large scale of symbiotic relationships ranging from benign and opportunistic to deadly and obligate, and may have little connection to whatever another person is calling a "parasite." The main obstacles to understanding of evolutionary relationships between hosts and parasites, therefore, are investigator ignorance and investigator agenda. The ignorance often limits an investigator's ability to put host-parasite relationships into their phylogenetic and/or a natural context; an agenda often drives a search for systems to further it.

(5) A fifth general rule related to parasitism is that systematics matter. This rule is the one often, if not typically, violated by people who are studying host-parasite systems in some agenda-driven manner. No matter what his/her interests, a biologist must respect the power of scientific names and understand clearly how those names are actually links to vast amounts of information, much of it of evolutionary importance. In addition, the names are the communication system by which we put our work into an evolutionary context. A scientific name carries with it a historical record, a set of observable properties, an assumed set of developmental events and requirements, an ecological niche, and a bunch of relatives, all of whom also have names. The word "parasite" is not a scientific name; *Haematoloechus coloradensis* is a scientific name; *H. coloradensis* is a parasite, but not all parasites are *H. coloradensis*. When people who understand the first four rules use the term *Haematoloechus coloradensis* in conversation, suddenly the evolutionary context is established, constraints on

and opportunities for transmission are understood, and participants in this conversation are able to screen out irrelevant information.

(6) A sixth general rule about parasitism is that the study of it, namely parasitology, is a highly integrated discipline. Thus all parasitologists understand that pathology, immunology, transmission, development, parasite genetics, physiological relationships between host and parasite, evolutionary history, geographic distribution, and the practical problems of diagnosis and identification, are all inextricably linked to one another. This understanding is the basis for parasitologists' breadth (usually forced on them by the discipline), although they all understand that specialization is necessary for a productive research career. You simply cannot study everything about even a single host-parasite system during a single lifetime.

(7) The seventh general rule is that the overwhelming majority of symbiotic organisms do not cause disease, or if they do, such disease is relatively mild compared to some other risks faced by a potential host. The risk of an embryonic or hatchling duck being eaten by a bull snake, for example, is many times, perhaps many orders of magnitude, greater than the risk of not mating, or not successfully completing migration a year later, because of a hundred tapeworms in its gut. So when assessing, or even contemplating, the potential damage that parasites can inflict on hosts, especially in an evolutionary context, it is important to consider the hosts' entire lives, including ecological requirements, predators, etc.

(8) The eighth general rule is that parasites are divided into two categories: microparasites and macroparasites. The former multiply inside the host individual in whatever developmental stage actually occupies that particular host. Malarial parasites, trypanosomes, amebas, and viruses are all good examples of microparasites. Macroparasites do not multiply inside the host in the developmental stage that occupies that host. Large roundworms and tapeworms are good examples of macroparasites; either group may produce staggering numbers of eggs that are subsequently passed in feces, but adult tapeworms typically do not produce more tapeworms within the original host. Whatever factors produce selection pressures on both hosts and parasites differ markedly between these two general types of parasites—micro- and macro-.

(9) The ninth general rule is that in nature, most host species are either uninfected with a particular parasite species, or are only lightly infected, and that most of the parasites are in a relatively few host individuals. This rule applies mainly to macroparasites, but with these kinds of parasites, it is the most important evolutionary consideration (again, see Figure 2).

(10) The tenth general rule is that in the case of parasitic organisms, persistence is the measure of success, not numbers. Thus when contemplating parasite properties, it is probably wise to begin with the life cycle, which is an inherited and evolved boundary condition, then ask how much reproductive potential must be maintained for the parasite species to persist in the long term. So any model that begins with assumptions about numbers must make sure that these assumptions focus on the life cycle steps that limit, or allow, persistence. This requirement for persistence as a measure of success means that host-parasite evolution

models must take into account the full range of ecological conditions under which hosts and parasites co-occur, including alternate or reservoir host species.

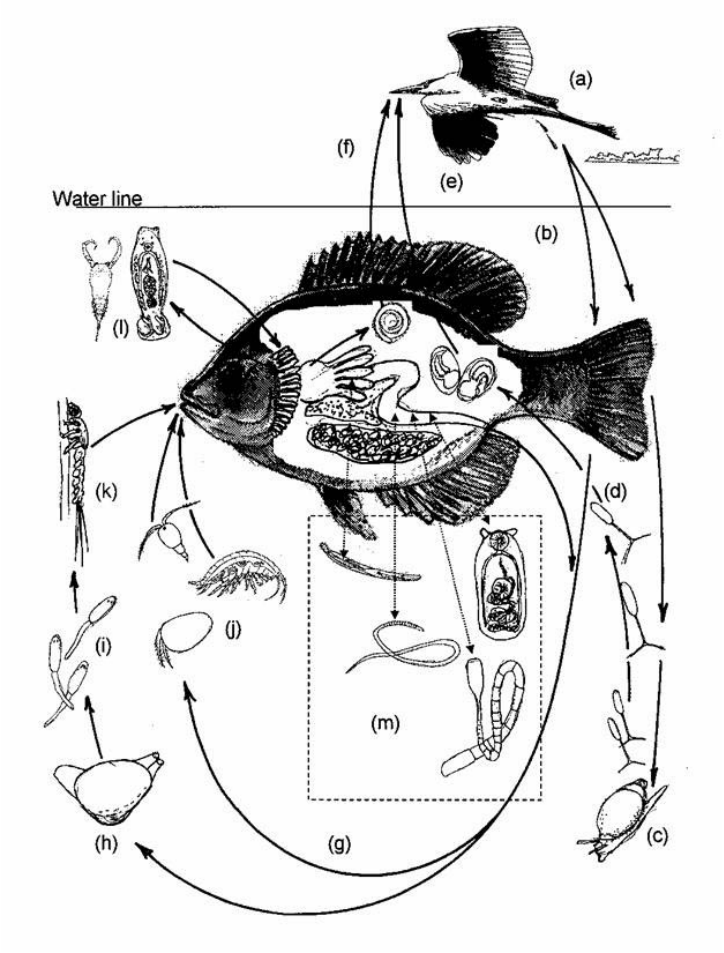


Figure 1. Ecology of parasitism in a typical North American freshwater pond.
(a) Fish-eating heron, the habitat of several adult helminth parasites that use bluegill as second intermediate hosts. (b) Trematode and nematode eggs being passed in heron feces and dropping into the water. (c) Snails that serve as first intermediate hosts for most trematodes. (d) Trematode cercariae emerging from snails; cercariae penetrating the fish, where they encyst as metacercariae. (e) Heron becoming infected with trematodes upon eating a fish containing metacercariae. (f) Heron becoming infected with a nematode upon eating the same fish that also contains an encysted juvenile worm. (g) Passage of helminth eggs from both heron and fish feces into the water. (h) Fingernail clams that first intermediate hosts for adult trematodes in bluegill. (i) Cercariae emerging from fingernail clam to penetrate and infect a mayfly nymph (k). (j) Community of small crustaceans serving as intermediate hosts for nematodes that live in both heron and fish, acanthocephalans from fish, and tapeworms from fish. (l) Ectoparasitic flatworms and crustaceans that occupy the fish's gills and have direct life cycles. Within the box (dotted line) are acanthocephalans from the fish's pyloric cecae, and nematodes, trematodes, and cestodes from the fish's intestine.

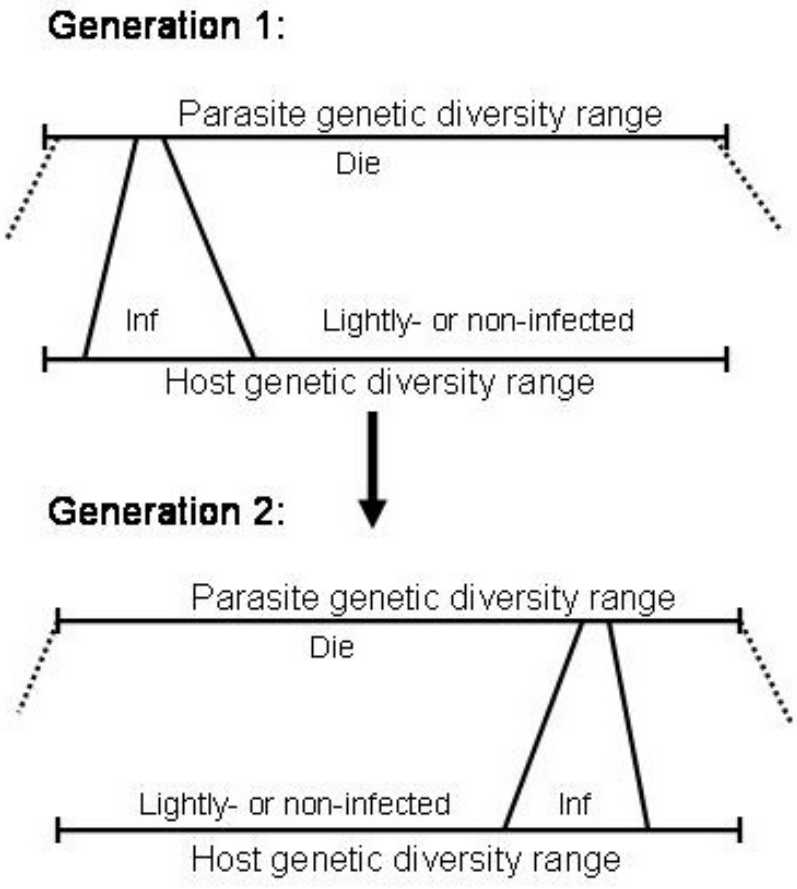


Figure 2. An illustration of the typical flow of parasites into hosts
Most parasites have enormous reproductive potential, but the probability of any one parasite surviving to infect another host is relatively small. In addition, within the vast majority of host species' populations, the majority, and often a large majority, are either not infected or are only lightly infected. The exceptions are, of course, the disease cases that we are inordinately interested in, and even in those situations, parasites are often distributed quite unevenly across time (thus the term "epidemic.")

7. Some Representative Life Cycles

An understanding of life cycles is critical to an understanding of parasitology. Life cycles are important to know because it is impossible to get an accurate picture of the way a parasite species exists in nature unless one is aware of the life cycle requirements. For this reason, parasitologists in general have great reverence for life cycle diagrams, and for the labor of love required to solve life cycle problems. In addition, regulation of a parasite population may occur at only one stage of the cycle. The presence or absence of a parasite species in a particular location may be a manifestation of the presence or absence, respectively, of some intermediate host.

The vast majority of parasite life cycles are simply not known, in the sense that the species which serve as intermediate hosts in nature have not been discovered, nor the intermediate stages of parasite development described. Therefore, a parasitologist is always on the lookout for observations that may hint at the life cycle in nature. Thus in the same sense as a parasite must be opportunistic, the parasitologist must also be that way, always alert for the chance to discover some bit of information that will lead to eventual discovery of the natural cycle. However, many life cycles have been completed experimentally using non-natural hosts. From these kinds of studies, as well as the cycles known in nature, come our willingness to speculate, based on extrapolation, as to the general features of unknown cycles.

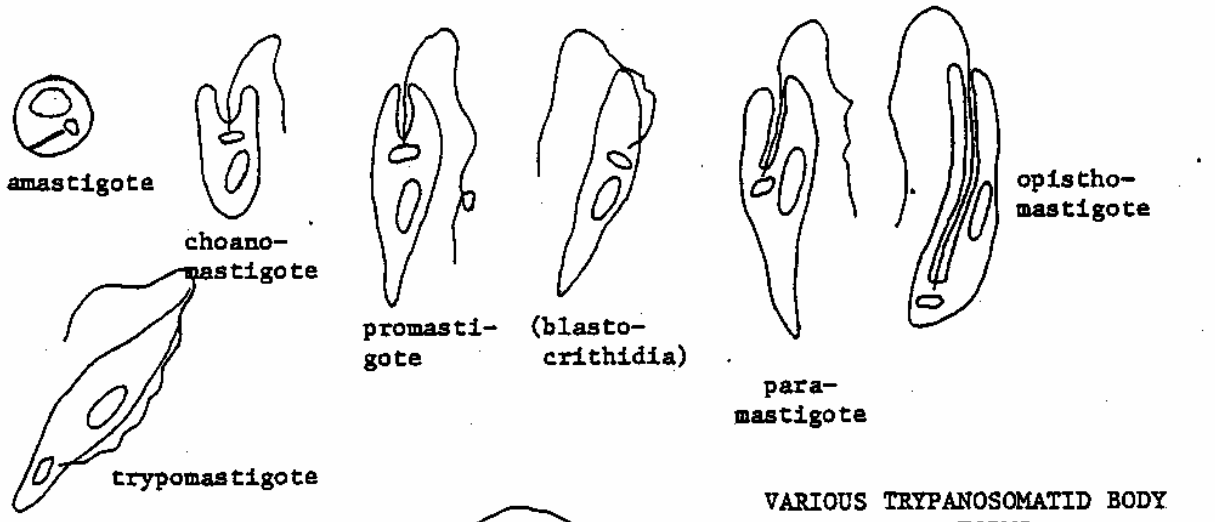
Your understanding of life cycles will grow most quickly if you acquire some definitions. An **intermediate host** is one which is required for the embryological development of the parasite but in which parasite sexual reproduction does not occur. A **definitive host** is one which is required for the development of the parasite, but it is also the one in which sexual reproduction occurs. Often intermediate hosts are invertebrates such as snails and insects (especially beetles, orthopterans and odonates), but rodents and lagomorphs serve as intermediate hosts for cestodes that mature in large predators such as coyotes and cats. **Paratenic** hosts are those in which parasites survive, and which often accumulate many parasites of a particular type, but which are not necessary for completion of the life cycle experimentally. Therefore paratenic hosts may play an important ecological role, or may even be essential for the completion of the cycle in nature, regardless of whether they are required for parasite development.

The life cycles illustrated in the next few pages are representative of a number of groups of parasites. Although they are diagrammed as a well ordered set of events, remember that in nature these cycles operate at the population level. That is, some fraction of the parasite population makes the transition from host to host. All such cycles are vulnerable to annual fluctuations in host populations, to physical factors which can influence transmission dynamics, etc. Thus you may find great differences, between locations or times, in the prevalence or density of parasites, simply because of the effects of abiotic environmental factors on the chances of a parasite completing the cycle.

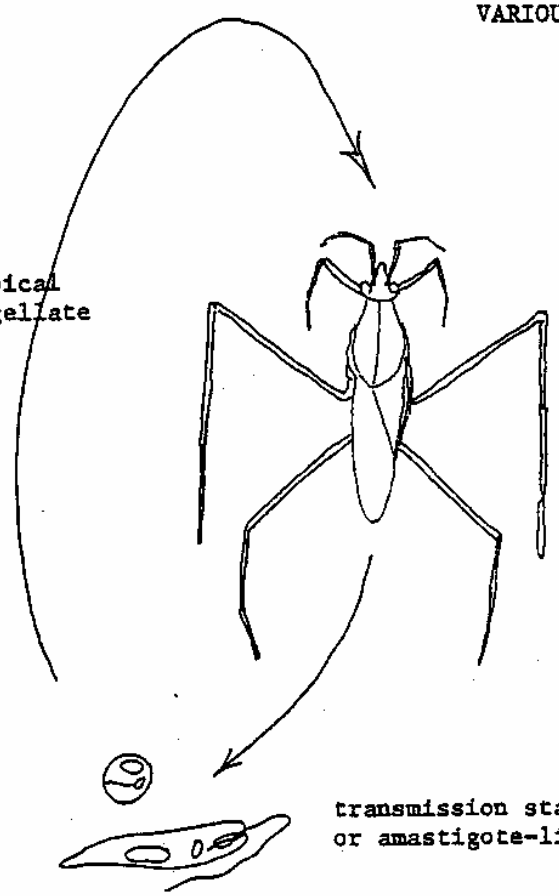
The best thing to do with the following life cycles is memorize them and then use them as a general set of background information. They are pretty useless as specific facts. They are

exceedingly useful as conceptual frameworks within which to place your field observations or to use in interpreting your field observations.

LIFE CYCLE – trypanosomatid flagellates



VARIOUS TRYPANOSOMATID BODY FORMS



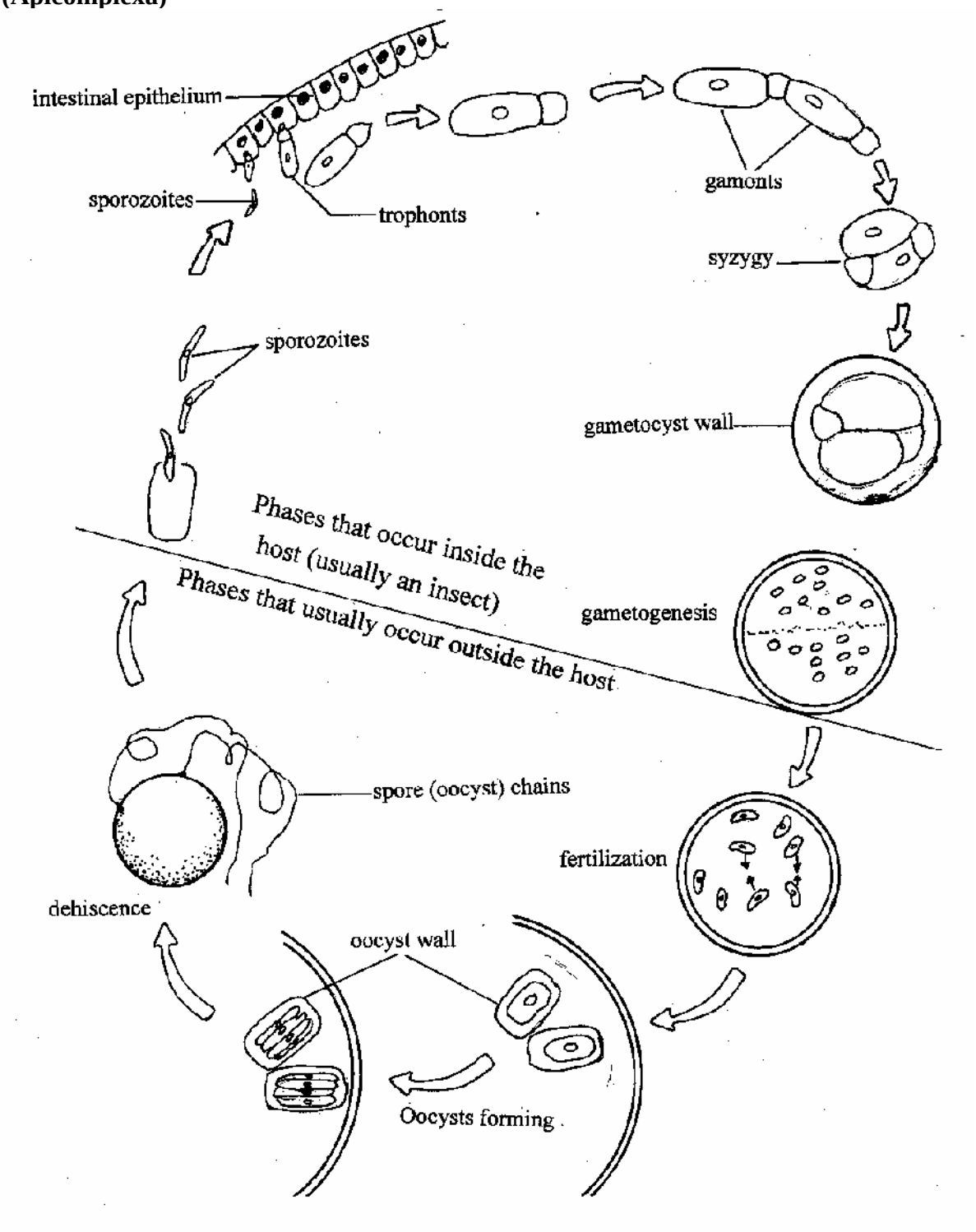
life cycle of a typical trypanosomatid flagellate found around CPBS

host is an insect

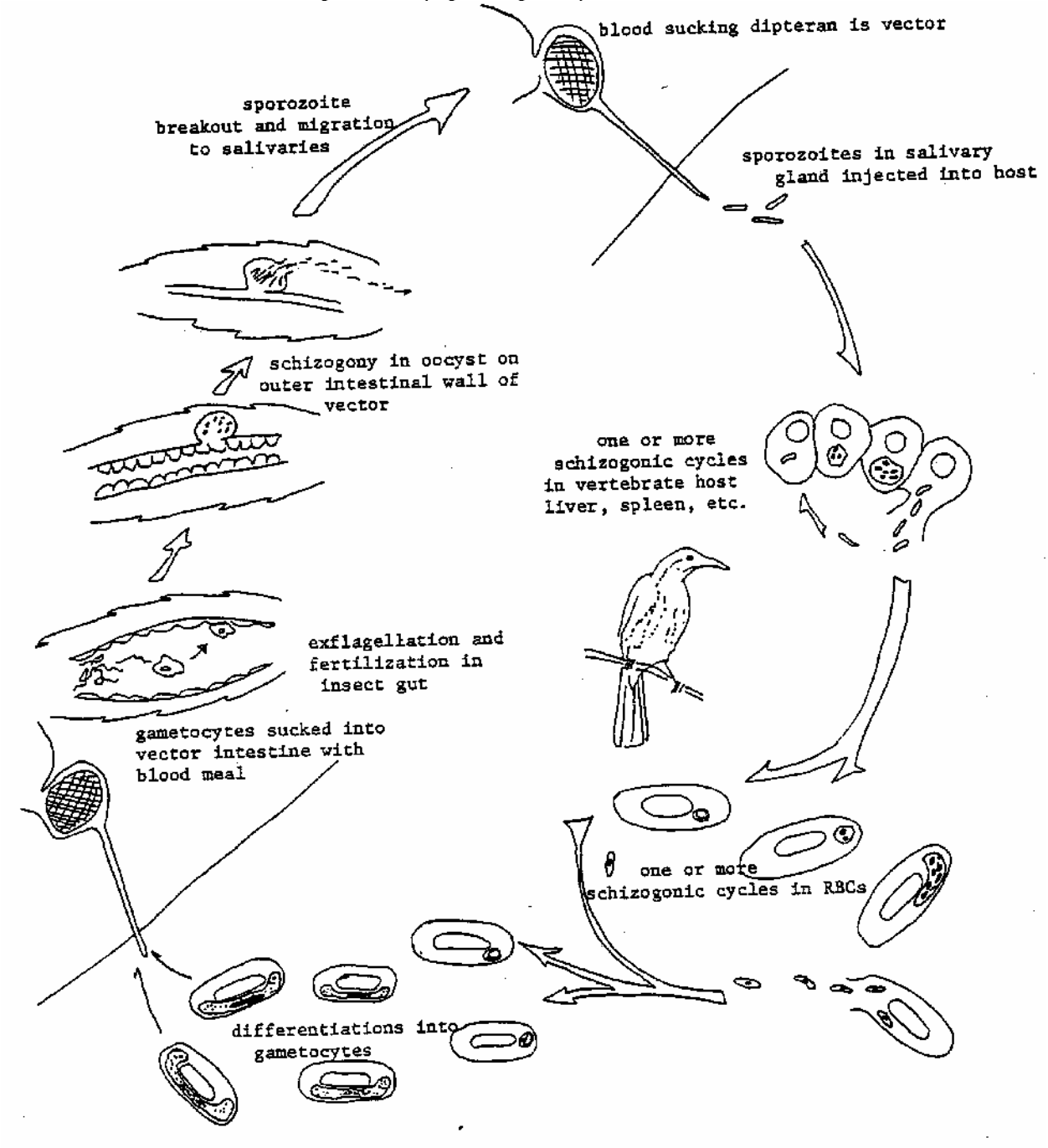
infection is intestinal

transmission stages may be promastigotes or amastigote-like "cysts"

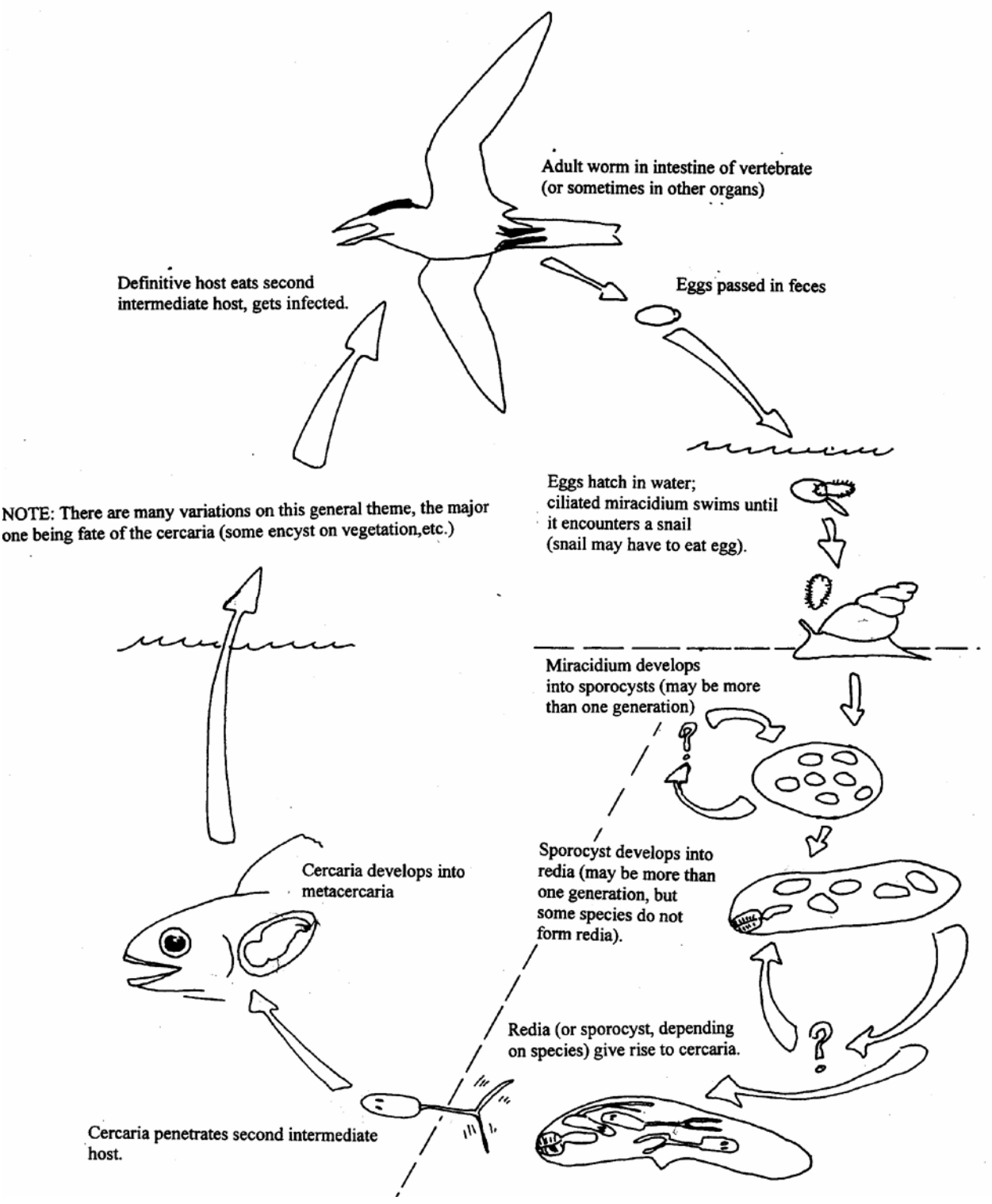
LIFE CYCLE – A typical gregarine (Apicomplexa)



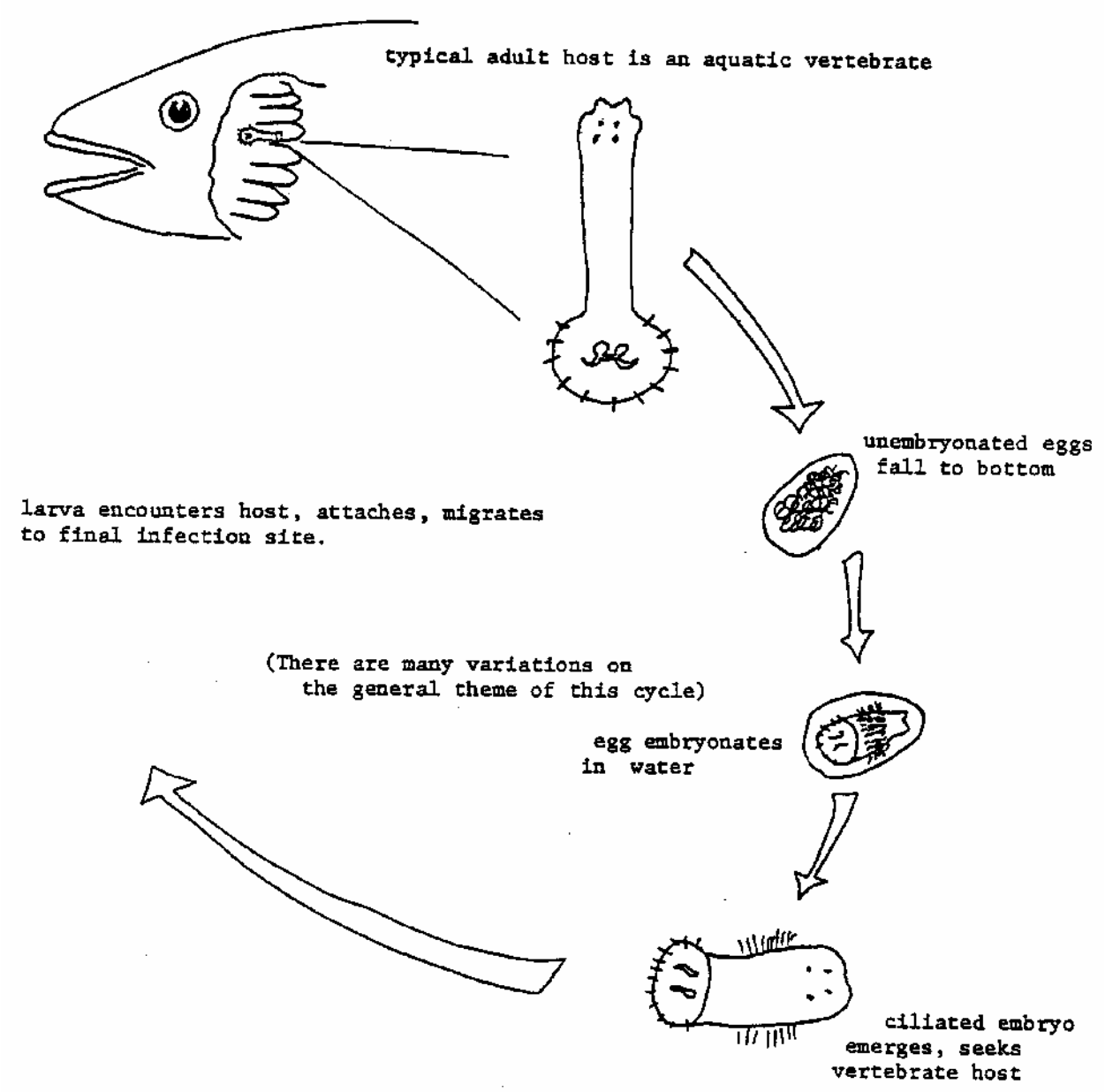
LIFE CYCLE – A malarial parasite (Apicomplexa)



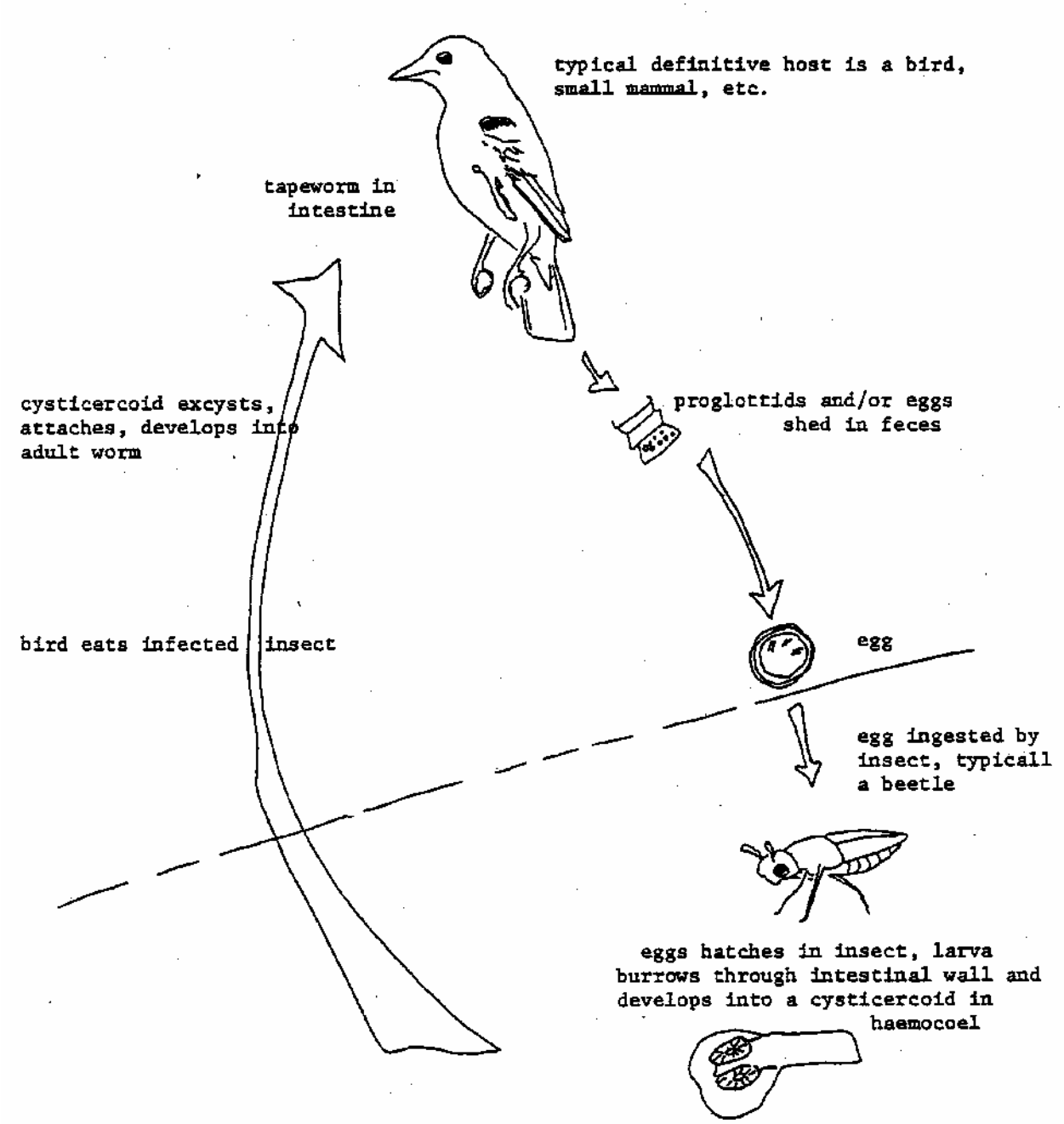
LIFE CYCLE – A digenetic trematode (Platyhelminthes: Trematoda: Digenea)



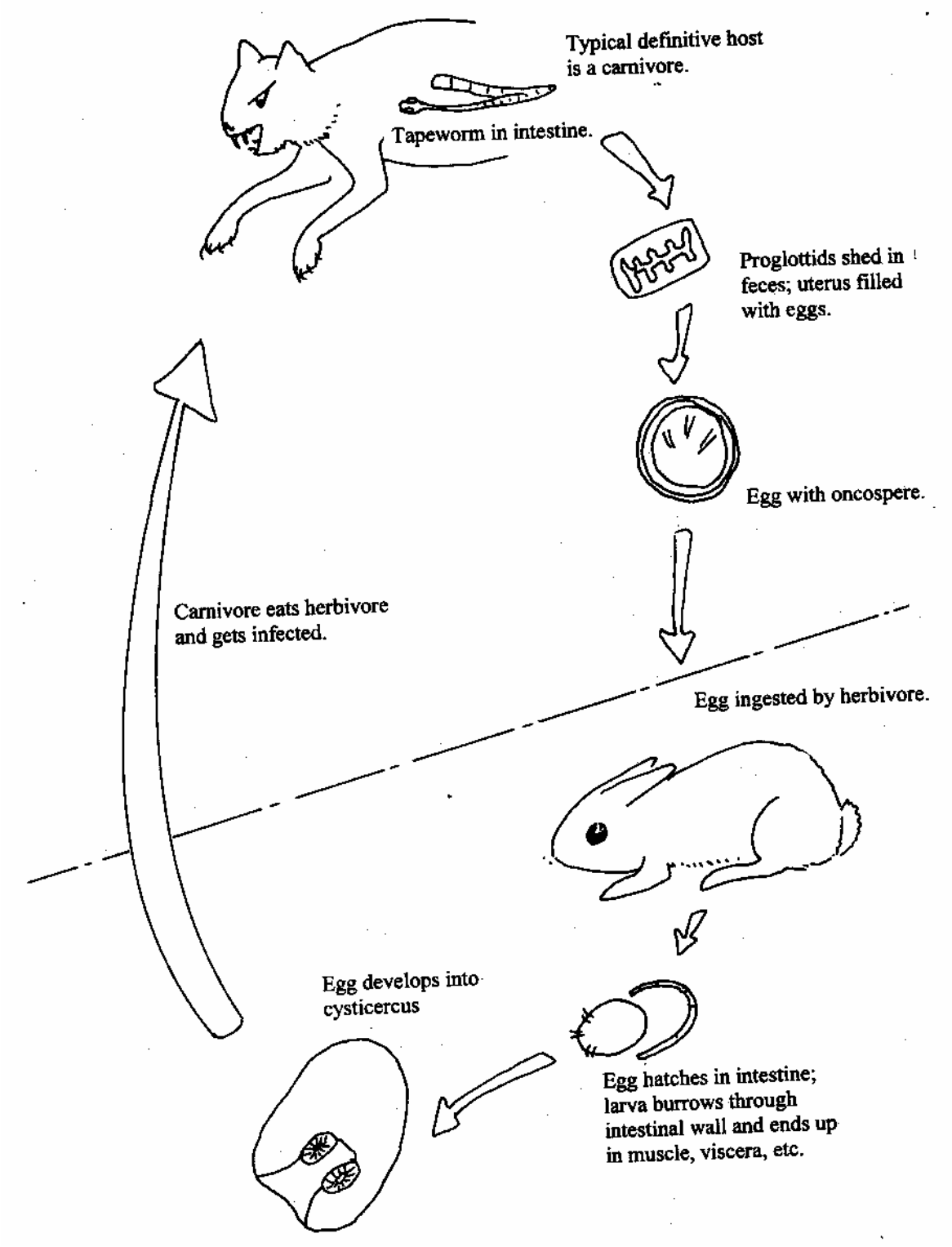
LIFE CYCLE – A monogenetic trematode (Platyhelminthes: Monogenoidea)



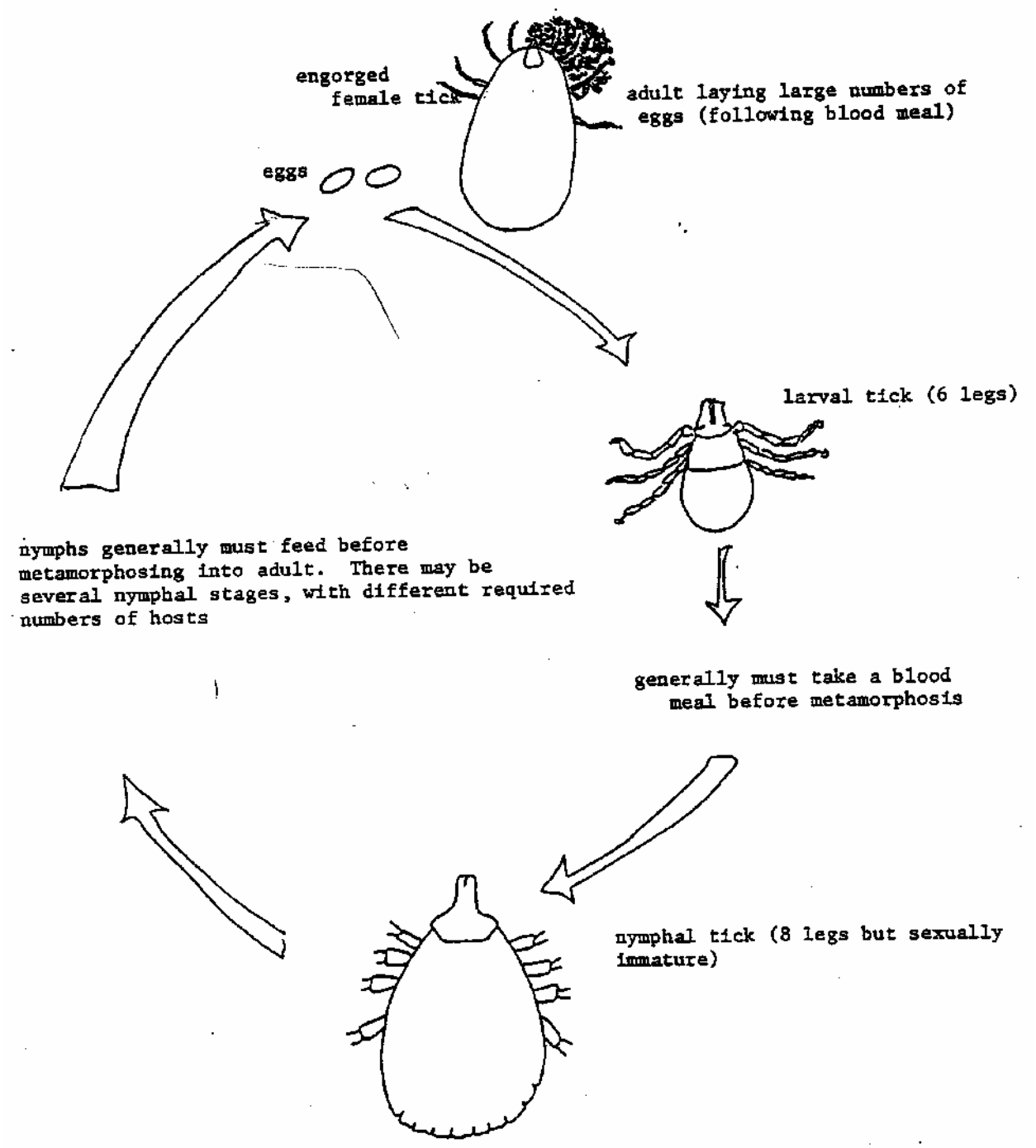
LIFE CYCLE – A hymenolepidid cestode (Platyhelminthes: Cestoidea)



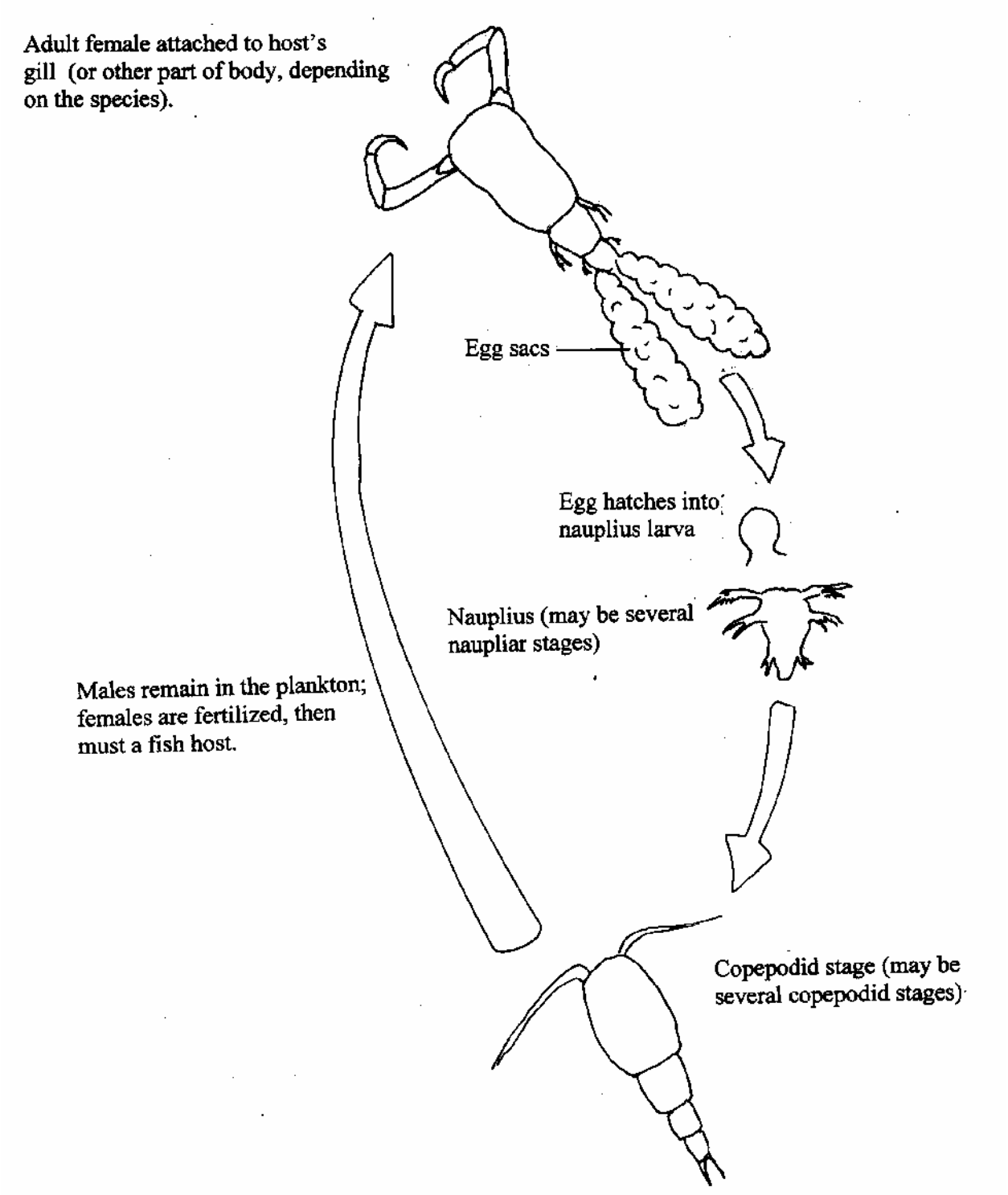
LIFE CYCLE – A taeniid cestode



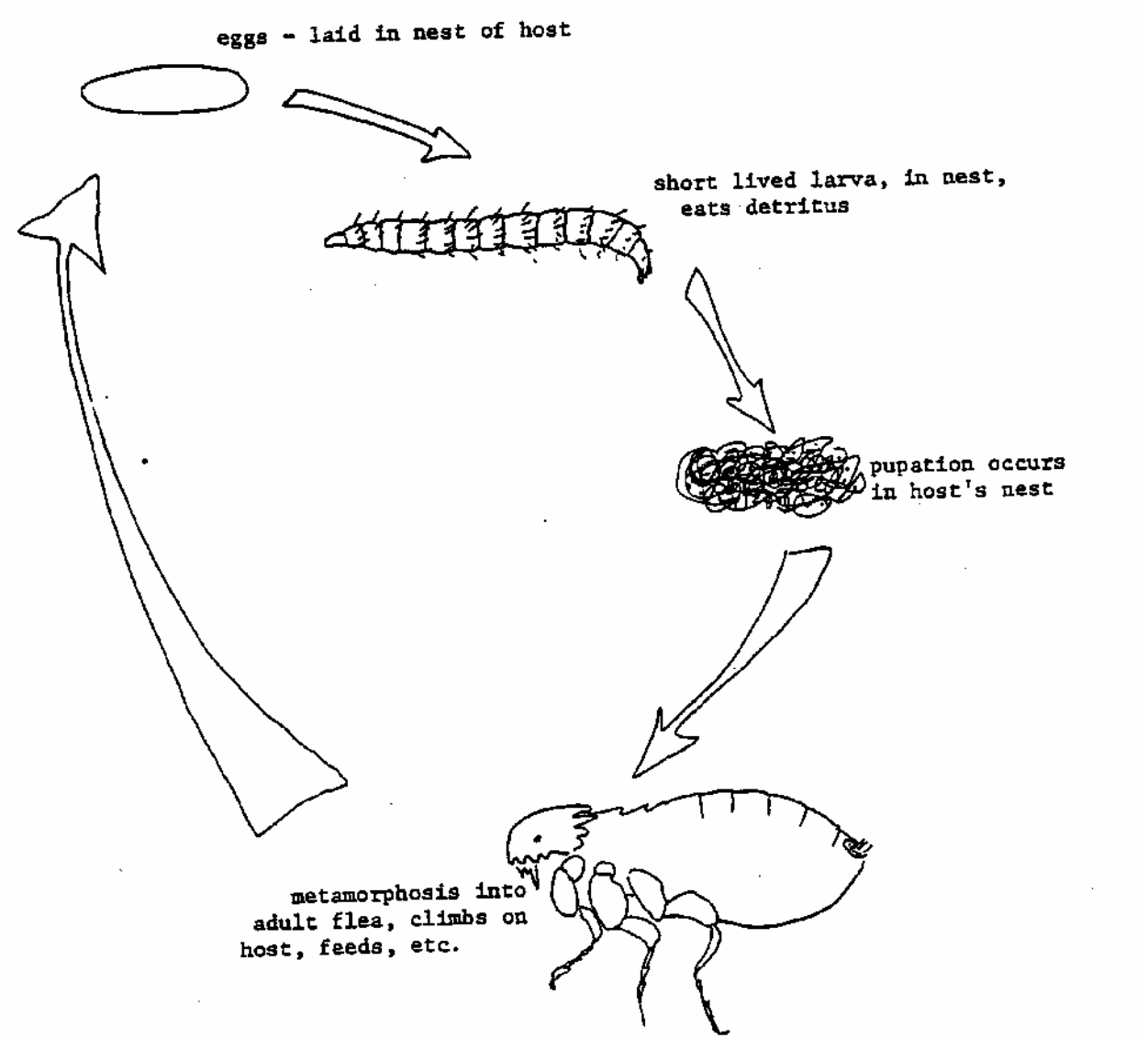
LIFE CYCLE – A tick (Arthropoda: Arachnida: Ixodidae)



LIFE CYCLE – A parasitic crustacean (Arthropoda: Crustacea: Copepoda)



LIFE CYCLE – A typical flea (Arthropoda: Insecta: Siphonaptera)



8. Hints on Dissection

Dissection of an animal for parasites will be demonstrated the first day of class. You will learn that there are complete dissections then there are functional dissections. Complete dissections are ones in which a serious attempt is made to recover every single parasite in/on a host. Not many people have the patience or skill to do a complete dissection, unless their research requires it, then they either develop the patience and skill or fail. Functional dissections are those in which adequate parasite material is recovered to do the lab exercise, which are done consistently enough to ensure that data from different students can be pooled, and which make an attempt to recover all parasites but do not subordinate the lab exercise to the recovery of every last worm or protozoan. I encourage you to be as thorough as possible in your dissections; if you purposefully ignore a place where parasites might be, then you've wasted a wild animal.

You could, at this point, be given detailed instructions on how to cut up an animal. Instead of doing that, I will usually demonstrate a dissection when I think such a demonstration is needed, and it usually is the first time we work on a particular host species. However, some general hints are in order. First, if you have an opportunity, any terrestrial vertebrate should be examined for ectoparasites: fleas, ticks, mites, lice, etc. This examination can be done by combing the feathers or fur "against the grain" over a white piece of paper or a white pan. Secondly, with any vertebrate, but especially amphibians and reptiles, you should actually look into the mouth. With fish, the gills should be examined under a dissecting microscope for copepods and monogeneans, and with a compound microscope for peritrich protozoa. The fins of fishes also have ectoparasitic monogeneans. Thirdly, the body cavity is usually opened with a mid-ventral slit; care should be taken not to rupture the intestine. In this way the body cavity itself, as well as organs such as lungs, gall bladder, urinary bladder, can be examined free of the intestinal mess. Finally, the intestine should be removed to a separate dish for dissection.

Digestive tracts can be cut up into pieces if you wish (esophagus, stomach, intestine) and the parts separated, although if you cut the intestine itself into pieces, you are likely to also cut intestinal worms into pieces, which may ruin them as specimens. Intestines are split longitudinally, with the inside scissors blade held against one wall, and gently washed out in a wax-bottom pan with water in it. It is always wise to pick up any suspicious item with a pipet and examine it under a dissecting microscope. Most helminth parasites are opaque or translucent white; many of them move; they range from big and dramatic to microscopic. Generally, students find them all quite fascinating and beautiful. It is indeed an unique and intriguing experience to actually find a worm inside another animal that you yourself have cut up.

Invertebrates are usually dissected with less care and success. There is a technique that works fairly well for pulling the intestine out of most insects, including larvae. Cut off the head, nick the sides of the last segment of the abdomen, put a probe on the thorax and another on the tip of the abdomen and gently pull. Do this in a little water on a slide, maybe under the dissecting scope. Ideally, the gut should come out in one piece. It is often full of protozoa. As an alternative, you might also try clipping off the posterior end, then pulling the gut out by forceps on the head. The body cavities of certain insects should always be examined. These insects include grasshoppers, roaches, crickets, beetles, and odonates, all of which may serve as

intermediate hosts for helminths of land vertebrates, or have parasitoids or even hyperparasites in them. Snails are often simply crushed. If you have never dissected a snail, however, try a careful dissection of the largest snail you can find. Again, carry out the dissection in water. A first hand look at snail internal anatomy will tell you immediately that there are worlds of things we don't learn in Lincoln in the average set of courses taken by a major.

I generally try to avoid using scalpels and razor blades. Use of such tools is usually the quickest way to dissect your finger. Finally, there will be times when other classes bring in animals, people bring back fish they've caught for eating, etc. Do not let an animal get thrown away without somebody checking it for parasites!!!! IF YOU SEE A FRESH ROAD KILL, PICK IT UP IF YOU CAN DO IT SAFELY AND BRING IT BACK TO THE LAB FOR DISSECTION!!!!

9. Specimen Preparation

The general philosophy and approach of specimen preparation:

The philosophy: Specimen preparation is an art that in turn is a pre-requisite to your science. In field work, as in molecular biology, the first question that must be answered is: What is it? The "it" in this case is the animal you wish to study. When this animal is a microscopic invertebrate, then your ability to identify it depends largely on your ability to prepare a specimen adequately. in Field Parasitology, we give you plenty of opportunities to prepare specimens. We do specimen preparation for a number of reasons, ranging from the idealistic to the practical. I honestly believe that any biology major simply must come to understand what is involved in describing the inventory of organisms that inhabit this planet, and to understand the role that museums play in maintaining the evidence for this inventory; thus the idealism. From a practical point of view, many of you will be health care professionals in a few short years, and you will discover rather quickly that specimen preparation is the first task that must be accomplished satisfactorily before a diagnosis can be made.

Some rules: Here are seven rules of specimen preparation. If you learn, remember, and follow these rules, then your chances of succeeding will be maximized.

(1) The first rule of parasite specimen preparation:

> ***Consider the activity an art and approach it in that manner.***

(2) The second rule of parasite specimen preparation:

> ***Make your mistakes early and often enough so that you can learn to correct them by yourself.***

(3) The third rule of parasite specimen preparation:

> ***Keep your stuff as clean as possible and use common courtesy and common sense in your handling of chemicals (also follow correct safety practices).***

(4) The fourth rule of parasite specimen preparation:

> ***Get the water and alcohol out of your specimen before you try to make a permanent slide preparation.***

The majority of specimens you will prepare end up mounted on slides in a synthetic resin that is insoluble in water or alcohol. However, organisms are mostly water and the fixatives you use to preserve them are made up in alcohol. Thus we have a problem: If your specimen is to be of any use, it must be freed of the alcohol and water before it is put on a slide. This removal must be done because light will not shine through your specimen, on the slide, if the water and

alcohol are "precipitated" in the mounting medium. Most or all of the seemingly complex set of procedures used in specimen preparation are simply ways of removing the water and alcohol from your specimen and replacing those chemicals with an organic solvent.

(5) The fifth rule of parasite specimen preparation:

If you stain your specimen, stain it all the way through, then de-stain the outside.

Parasites are most easily studied if they are stained. Stain is of the most help when it is present in the internal organs and absent in the integument, thus enabling you to see through the parasite's surface and into the interior. Many if not most of the taxonomic and identification characters we will use for some species also involve internal anatomy as revealed by stains. But animals differ in a variety of ways, not the least of which is the nature of their outside coverings. In practice, therefore, you accomplish this staining feat by staining the specimen all the way through, then removing the stain from the outside. Differences in specimen handling result from variations in the specimens' outside coverings.

(6) The sixth rule of parasite specimen preparation:

The specific techniques you use, and the way you use them, will depend somewhat on the kind of parasites you have.

Variations in staining techniques are related mostly to specimen size and outside coverings. You can do things with small specimens, for example, that would be a disaster with larger ones; and, you can do things with soft worms that you can't do with insects or ticks.

(7) And the final rule:

Large parasites present different problems from small parasites. (Arthropods present different problems from helminths; protozoans present different problems from either arthropods or helminths, etc.)

Methods:

The following methods are a guide to accomplishing the above tasks. They are simply detailed instructions for obeying the rules.

Slide marking: Mark each slide with a glass marking pencil with a code consisting of your initials, the slide number, and the year. For example, my first slide of 2000 would be marked JJJR-1-00. After marking, clean your slide with Windex® before using it to make a permanent preparation. Example:

Records: Collections are worthless and are not accepted without a completed record sheet. Each specimen you turn in for a grade must also be accompanied by a 3x5 card, made out according to the following example:

KMJ-2-88

HOST: Pimephales promelas
PARASITE: Trichodina sp.
COLLECTION SITE: South Platte River, Roscoe, NE
INFECTION SITE: Gills
DATE: June 17, 1988
COLLECTOR: Kris M. Jones
REMARKS: Heavily infected

NOTE: We will also add latitude and longitude coordinates to these collection records.

Steps in making permanent slides:

* = steps at which preparation can be interrupted or specimen stored.

BLOOD AND TISSUE PROTOZOA:

1. Make a thin smear on a clean slide.
2. Air dry.
3. Fix for 1 minute with absolute methanol.
4. Stain with Giemsa stain.
5. Study under oil immersion without coverslip (no need for coverslip).

GREGARINES AND LARGE ECTOCOMMENSAL PROTOZOA:

1. Make a “gut drag” with no water, on clean slide.
2. Air dry slide.
3. Fix and stain in Semichon’s acetocarmine, 3-6 min.
4. Dehydrate through ethanol series, 3 min per concentration.

5. Clear in xylene, add mouting medium and coverslip.

PLATYHELMINTHES:

1. Relax in tap water in refrigerator or at room temperature 1 hr to overnight as required; tapeworms can be killed in boiling water.
2. Fix in AFA - 15min - 2 hrs, depending on size.
3. Soak in 70% EtOH to remove AFA (30 min-overnight).
4. Can stored in 70% EtOH.*
5. Stain heavily.
6. De-stain with acid alcohol so that inside is still dark and outside is light.
7. Dehydrate through ethanol series of increasing concentration.
8. 2-3 changes of 100% EtOH.
9. 1:1::EtOH:xylene.
10. Xylene.
11. Mount in mounting medium.

ACANTHOCEPHALA:

1 - 4. Same as above except puncture body with an insect pin.
5 - 11. Same as above except that you will have to leave the specimen in all solutions much longer than with a small platyhelminth.

LEECHES:

About the same as for acanthocephalans, but my best advice is to learn the tricks of the trade from a veteran leech person. There are a number of tricks to get them stretched out, but without the use of narcotics, these tricks are not always successful.

NEMATODES:

1. Fix and straighten with glacial acetic acid. If small, can puncture and treat as acanthocephalans. If large, you're sort of on your own.

ARTHROPODS:

1. Place directly in 70% EtOH or AFA from host.
2. If specimens are large and/or dark, bleach in sodium hydroxide.
3. Specimens like ticks will have to be punctured with an insect pin so that the bleach dissolves out the insides.
4. Soak in 70% EtOH to remove bleach.
5. May store in 70% EtOH.*
6. Dehydrate and mount in mounting medium as with acanthocephalans.

MISCELLANEOUS OTHERS:

Try something intelligent and see if it works.

10. Field Notes

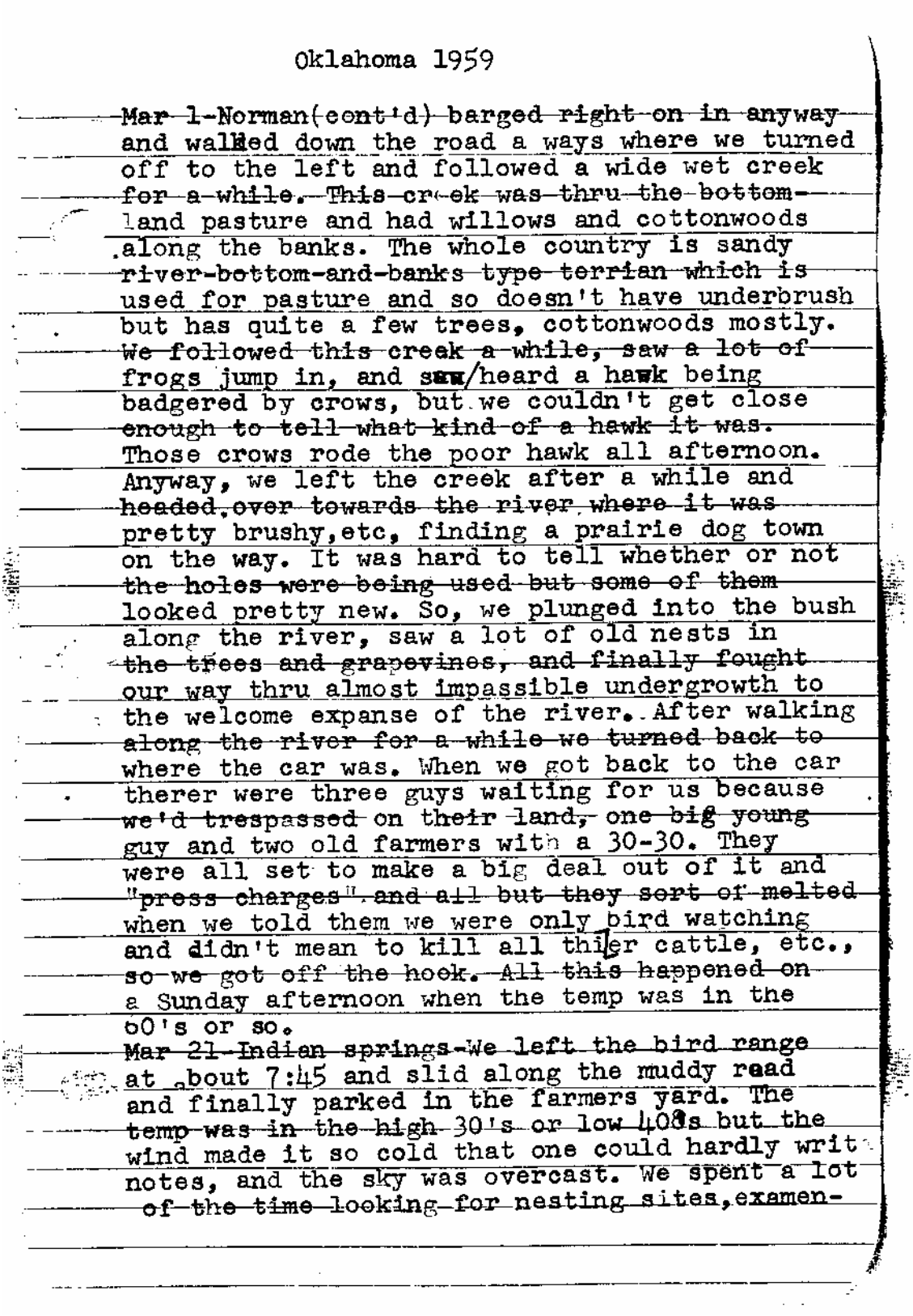
Some years I ask for a set of formal field notes; or, you may wish to begin a set for your own reference, pleasure, memory, etc. I offer the following suggestions about such notes. Field notes are exactly that: field notes, not book notes. That is, an exercise in taking field notes is really a means of forcing you to write down what you see, rather than what somebody else tells you to see. I have found that field notes are most useful when then are written in two separate parts, a daily log or journal, and a species record. Both should be loose leaf.

(1) The daily log, dairy, or journal portion is where you describe, with chronological entries, the weather, locations visited, circumstances of the field trip, people who accompany you, unusual observations about the physical surroundings, observations to help you interpret the biological part of your trip, etc.

(2) The species record is that portion of the notes in which you enter biological observations of each kind or organism you observe. The species section can be organized in any manner you wish - beginners tend to do it alphabetically, veterans phylogenetically. You may not have the full scientific name of every kind of animal you observe or wish to remember. Consequently, you may wish to start organizing by family names, sometimes even common names, but eventually you will want to look up the scientific names if they can be determined with certainty. In this section you have a separate page for each organism. On each page write, chronologically, your original field observations for a particular day. In this way you should be able to build a surprisingly large picture of the natural history of organisms you observe. Some pages will have a great many entries, others only a few.

This kind of notebook is pretty useless unless you work on it every day, making formal entries from your scribbled notes made in the field. I have enclosed a couple of pages from my own field notes from years ago as samples. The course was ornithology, not parasitology, and the place was Oklahoma, not Cedar Point. But the method remains the same.

An example of the log section of some field notes:



Oklahoma 1959

Mar 1-Norman(cont'd) barged right on in anyway and walked down the road a ways where we turned off to the left and followed a wide wet creek for a while. This creek was thru the bottom-land pasture and had willows and cottonwoods along the banks. The whole country is sandy river-bottom-and-banks type terrian which is used for pasture and so doesn't have underbrush but has quite a few trees, cottonwoods mostly. We followed this creek a while, saw a lot of frogs jump in, and saw/heard a hawk being badgered by crows, but we couldn't get close enough to tell what kind of a hawk it was. Those crows rode the poor hawk all afternoon. Anyway, we left the creek after a while and headed over towards the river where it was pretty brushy, etc, finding a prairie dog town on the way. It was hard to tell whether or not the holes were being used but some of them looked pretty new. So, we plunged into the bush along the river, saw a lot of old nests in the trees and grapevines, and finally fought our way thru almost impassible undergrowth to the welcome expanse of the river. After walking along the river for a while we turned back to where the car was. When we got back to the car therer were three guys waiting for us because we'd trespassed on their land, one big young guy and two old farmers with a 30-30. They were all set to make a big deal out of it and "press charges" and all but they sort of melted when we told them we were only bird watching and didn't mean to kill all thier cattle, etc., so we got off the hook. All this happened on a Sunday afternoon when the temp was in the 60's or so.

Mar 21-Indian springs-We left the bird range at about 7:45 and slid along the muddy road and finally parked in the farmers yard. The temp was in the high 30's or low 40's but the wind made it so cold that one could hardly writ notes, and the sky was overcast. We spent a lot of the time looking for nesting sites, examen-

An example of the species section of some field notes:

Turkey vulture
Cathartes aura (Linnaeus)

April 11-Newcastle road-On the way to the El Reno game farm we saw the dead one lying by the side of the road. It was fairly well beat up but Ernie and I pulled out a wi primary wing feather anyway for our collections.
April 26-Mount Scott-In the Wichita mountians wildlife refuge we saw many soaring around the mountians.
May 9-Arbuckle Mts.* Ernie, Ron, and I found the nest today. We were walking along the top of a ridge and the whole class saw the bird fly from behind a tree low to the ground across a deep gully. We three climbed down and up the canyon while members of the class directed us to the right tree. The "nest" was in a crevice which was about a foot and a half wide and about three feet deep and which widened out below the ground The two young were just sitting on the bare ground and there were remains of some egg shell around. The young were covered with furry down different from a hawk. They were white with black bald head and black chicken-like feet. They were terribly cute and reminded me of an old- old half bald colored man with white hair. They had a humanly old and wrinkled head and face. There was no down on the crop or lower belly. They would shake their heads at regular intervals as if frightened. The adult circled fairly close but never got within 100 feet. The young also held their little wings out sideways all of the time. Sutton said they were about ten days old. The crop was partly filled.

1960 Oklahoma
Oct 15-Regan trip-There was one circling in the group with the black vultures over Lowrent lake.

11. Data Collection

The major things to remember about data collection are:

(1) it should be done regularly, and

(2) it should be organized at the time of collection with its final analysis in mind.

In other words:

(1) don't wait until the last week of the session before starting to make observations on your project, and

(2) have some idea what kind of observations you will end up with, and therefore what kind of analysis you will need to perform, in order to test your hypotheses.

These two basic points are generally a mystery to people unless they've already been through one research project in which they've screwed up a year's work and had to repeat it. The points need not be a mystery. Just remember that when you select a project, and write an hypothesis, the next step should be a decision about the kinds of numbers you will need at the end in order to adequately test this hypothesis or confirm (or deny) a prediction you may have made. That is, just exactly what do you need to know in order to answer your question?

I recommend the design and construction of data sheets for many of your projects. We will use data sheets regularly during class exercises and you will become very familiar with them. A data sheet is nothing more than a form to fill out that forces you to gather the observations you've decided you need and put them down on paper in a way that will allow you to make sense out of them weeks later. In general, each research project will need to have its own, especially designed, data sheet. I encourage you to begin on this design as soon as you decide on your project.

As a minimum, the data sheet should contain a place to enter date of collection, host species, host age, age category, sex, and life cycle stage (if discernible), collecting site in latitude and longitude as well as section, township, and range, numbers of parasites of various species, locations in or on the host's body (if relevant to the hypothesis), sex and life cycle stage of parasites (if discernible and/or relevant), and a place for remarks. Ideally, each host individual should be numbered, and research records should be retrievable by individual host.

Data sheets are typically filled out at the time of dissection, measurement, or counting. One of the best ways to make a mistake, or lose hours or days of work, is to jot down data on a paper towel or scrap of paper, intending to enter the numbers later. I *strongly* encourage you to make your data sheets, and data collection routines, a regular part of your research activities, and to fill in the measurements or counts as they are made. Personally, I tend to use pencil for data sheets, mainly because pencil does not run when it gets wet. Ballpoint is generally to be avoided because it does run when it gets wet.

You are likely to want to make a spreadsheet, too, that allows you to analyze your data in various ways, send it to statistical software, or use as a plotting device. If you design your spreadsheet exactly as your paper data sheet, then you will find it easy to transfer numbers at the end of each working day. From past experience, I routinely make the columns the attributes counted or measured, and the rows are the individual parasites or hosts in the sample. You can also easily add columns for codes, e.g. date, site, or other attributes of a host or parasite, and later sort according to those codes. You should always keep both hard and soft copies of your data files, as well as your original data sheets, even for projects that are still in progress, and make sure the copies are separated so that if you lose one, the chance of losing the other at the same time is minimized. I tend to always make at least two disc copies of research spreadsheets and keep them physically separated in different buildings.

Finally, write legibly. Make your entries as if they will be read, and the complete research project reconstructed years hence, by a stranger writing your biography after you become famous and bequeath your papers to a museum archive.

12. Parasite Ecology - Some Fundamental Concepts

(1) Trophic relationships: All parasites require their energy in the form of existing complex carbon molecules and require their nitrogen in the form of a mixture of amino acids. Thus parasites are no different nutritionally from free living animals. Parasites' feeding devices, however, may differ considerably from those commonly seen in other animals.

Parasites are always at a higher trophic level than their hosts. In some cases this trophic relationship is blatantly obvious: a trematode larva embedded in the tissues of a snail, an intracellular protozoan. In other cases you may have to become a philosopher to justify in your own mind the fact that a parasite is at a higher trophic level than the host. For example, a significant part of the host's energy used by the parasite is used in regulation of the environment. In other words, the parasite may by thought of as "preying" on the homeostatic mechanisms of organisms at lower trophic levels.

In nature, the overwhelming majority of parasitic relationships are not obviously disease relationships. Disease may be the exception rather than the rule, but subclinical detrimental effects on the host have been demonstrated in a variety of systems, e.g. in influencing the success with which male guppies attract females. Theoretically, such effects have the potential to in turn direct evolutionary changes in the host population. There is an oft-stated principle of parasitism that says it is in the best (evolutionary) interest of the parasite not to produce a pathological condition in the host. This particular dogma is one you may want to explore in your own thinking about parasitic relationships.

(2) The parasite's niche: A parasite's niche consists of its distribution on resources provided by the living body of another species of animal. Many parasites have life cycles with discrete stages, however, and some of these stages may be "free living" in the sense that they do not actually require a live host individual. Often these "free living" breaks in the life cycle are transmission stages, e.g. tapeworm eggs, coccidian oocysts, etc. Thus while a tapeworm egg can be considered a free living interlude, with a defined ecological niche (it is possible to set up environmental conditions in which the egg would not survive), that niche is sometimes a strange combination of breadth and poverty. Resources required for a host's niche may not be required for a parasite's "free living" interlude (tapeworm eggs don't eat grass; cows do). But the dimensions of a parasite's free living interlude on some abiotic resource may be exceedingly wide. So a parasite species' ecological niche may be thought of as a composite of discrete subniches, some of which correspond to the bodies of intermediate and definitive hosts, and some of which are broad but impoverished niches of transmission stages.

Every ecological principle, concept, etc., that you ever learned can probably be applied with little if any modification to the life of any parasite at any one life cycle stage. The only qualitative jump you might have to make is one in which you consider the parasite as a predator on the host's homeostatic mechanisms. Each stage's subniche can be defined as a hyperspace described in terms of dimensions on resources, each stage will have a population that is

distributed among ephemeral environmental patches, the host population will have a carrying capacity, some factors will work to regulate the parasite population (usually at a level below the carrying capacity), a parasite energy budget could theoretically be constructed, etc., etc. The parasite species niche, however, is still a composite of discrete niches of each life cycle stage.

(3) Resources, niche breadth, niche overlap: The ecological resources for a parasite are the same as those of potential hosts, e.g. space, time, temperature, osmotic pressure, concentrations of various molecules, measures of nutrients, etc. The problems of picking resources to actually study, in parasitology, are the same as those in other fields: a few such as space, time and temperature can be measured with some satisfaction; many others cannot. One general idea that will evidently not die is that of competition between parasite species, resulting in competitive exclusion or resource partitioning, within the body of a host. Stated more simply, some parasitological theoreticians assert that "parasite communities can be interactive." Others feel that parasite species do not compete with one another inside a host, and that the number of species and individual parasites present are largely a function of the probability of encounter. Those who study hosts that harbor many species of worms (e.g. ducks) typically consider parasite assemblages interactive. Those who study less parasitized hosts, e.g. fish, tend to think otherwise.

Regardless of how one views parasite assemblages (communities), as interactive or non-interactive, there is one very practical problem in dealing with assemblages as arrays of species distributed on resources. That problem is: the various parts of a host's body may not be units of the same resource, nor may they be easily quantified linearly. Stated simply, it is fairly satisfying to measure the dimensions of some parasite species on linear resources such as time and temperature, or even intestinal length. However, it is not very satisfying to consider eyeballs, peritoneal cavity, intestinal lumen, gill surfaces, skin, all as units of the same resource. While they are certainly all parts of the same resource in very general terms, e.g. a fish, they nevertheless are not obviously linear with respect to one another.

There are some obvious cases in which niche breadths and overlaps can be legitimately studied. The easiest of these cases in one we use routinely in a class exercise: the distribution of ectosymbionts on the surface of microcrustacea. Most of these ectosymbionts are protozoa that are not too difficult to distinguish, one from another. Microcrustacea are conveniently divided by Mother Nature into units of linear space, namely body segments. The symbiont assemblage can be analyzed according to the methods given in Chapter 11; evidence of competitive exclusion can be derived from niche overlap values. Speculation on the basis for potential interactions between symbiont species, on the other hand, opens up areas of the mind not normally used.

(4) Parasite populations: Parasite populations are generally distributed among hosts in an aggregated, manner. Thus for any one parasite species, most of the hosts are not infected, and the majority of the parasites are in the minority of the host population. This fundamental concept was developed using helminth parasites in vertebrates, and in fact using life cycle stages which did not multiply as that particular stage. The principle may not hold for parasite species that actually reproduce in or on the host, e.g. mites or blood protozoa. But regardless of the manner in which a parasite population is distributed among the hosts, you should assume that

there is some set of factors which determines the shape of the distribution, its mean, variance, etc.

For the purposes of Field Parasitology, we assume that distributions which differ in shape, i.e. in the type of mathematical equation that best describes them, are produced by different sets of encounter dynamics. In practice, we can ask: do the parasite/host encounter conditions vary with host age, sex, species? Does host species A encounter parasite species B in a specific manner in locality C, but is that specific manner different when A and B are found in locality D or F? These kinds of questions are very basic, general, overriding ones that dictate the directions of research. They are also the kinds of questions that can often be answered very easily by FP students using the right material.

(5) Parasite assemblages (communities): We will refer to the species of parasites occupying a host species as an assemblage, a term that implies the presence of several species, but does not imply interspecific interactions. The use of this term is a personal choice, and in fact, the literature on parasite assemblages refers to these groupings most commonly as communities. An immediate observation you will make is that some hosts support a more diverse assemblage than others. The diversity of a parasite assemblage can vary substantially from host to host, or host species to host species, or host sample to host sample, as a result of factors that do not alter the total resources available to the parasite. That is, diversity may be controlled more by abiotic factors than by biotic ones, even though the parasite's niche is by definition a biotic one. Thus our local observations will be a result of the availability of intermediate hosts, the extent to which weather conditions allow or promote transmission, etc.

(6) Geographic dispersal: It is characteristic of parasites that they tend to be dispersed over wide geographic ranges at fairly low taxonomic levels. Do not be surprised if you tentatively identify a species of worm as one that was described in India or Russia. This observation probably means that the host itself is fairly widely distributed, or has contact with related hosts which are widely distributed, or both. The geographic distribution of a parasite is tied to that of the host, but don’t think for a minute that fact hinders parasite dispersal on a grand scale.

Complex life cycles can multiply the potential mechanisms of geographic dispersal. The word "mechanism" is a key word: hosts for different life cycle stages may have quite different zoogeographic motilities (vagility). Thus in an evolutionary sense, the parasite with a complex life cycle also has multiple potential routes of dispersal. This is the basis for the restriction on the import of wild snails into the United States. The problem for the parasite, of course, is to find a suitable host at the new location. If the dispersing host is a vertebrate (e.g. a migratory bird), then a parasite requiring an insect intermediate host will not survive unless a suitable species is encountered. These problems are not as absolutely difficult as one might suspect. A surprising number of parasite species exhibit less than total host specificity, especially at the intermediate stages. Thus a single species of snail might serve as intermediate host for many species of trematodes.

(7) Host specificity: Most parasite species exhibit some degree of host specificity, being restricted to a narrow range of related host species, or sometimes a single one. This restriction is often most severe in the host in which sexual reproduction occurs, i.e. the definitive host. In addition, most parasites also exhibit tissue or infection site specificity.

In nature, both physiological and ecological specificity exist. In the first instance, a parasite species is restricted to a host(s) for biochemical or physiological reasons. In the second case, the parasite may be capable of infecting a wider range of hosts than it does, but is restricted because it does not have access to all potential host species.

(8) Reproduction: Some parasite species exhibit enormous reproductive potential, not only in terms of egg production, but also as polyembryony, or multiplication of the embryo (= larval stages). Combinations of the two mechanisms occur. In addition, reproduction may be phased so that it coincides with the highest probability of transmission. One idea that has been expressed by parasitologists is that relief from the physiological burdens of regulation has allowed parasites to invest a relatively large amount of energy in reproduction. Another idea you may encounter in the literature is that parasitism is such a chancy way of life, that high reproductive output is essential to survival. Feel free to wrestle, mentally, with these ideas and to determine whether your observations are consistent with them. Keep in mind, however, that parasites do not necessarily produce more parasites in exactly the same sense as cows produce more cows; instead, parasites tend to produce infective stages.

(9) Predation: Hardly anything preys on parasites. For those parasites transmitted orally, however, predation would be a blessing. It is possibly a fundamental concept of parasite evolutionary ecology that parasitism results in the avoidance of predation, and those species that require predation as an act of transmission sometimes exhibit features that promote it.

13. Quantitative Parasitology

The purpose of this section is to illustrate some methods of presenting and analyzing data typical of that obtained from parasitology field exercises. The quantitative tools and methods below are not necessarily unique to parasitology, but are particularly useful in summarizing, handling, and talking about results of parasitological studies. The terms, however, can be fairly unique to parasitology, and the problems of terminology have been addressed in the paper by Bush et al., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol*., 83:575-583.

Terminology: Here is a table of vocabulary words used in papers dealing with parasite populations and communities:

Ecological term	**Definition**
Population structure	A plot, along with the calculated quantitative descriptors, of a parasite population among hosts of a single species in an ecosystem.
Frequency distribution	A plot of the number of hosts of a single species (Y axis) vs. the parasite/host classes (X axis)
Infrapopulation	Number of parasites in a host (can take the value of zero)
Mean	Average number of parasites per host in a sample
Intensity	Number of parasites in an infected host (cannot be zero)
Metapopulation	All the infrapopulations in a single host species in an ecosystem
Suprapopulation	All the parasites of a species regardless of developmental stage, in an ecosystem
Infracommunity	All the parasites of all species in an individual host
Compound community	All the parasites of all species in a sample of hosts of a single species in an ecosystem
Prevalence	Fraction or percentage of a single host species infected at a given time
Incidence	Number of new infections per unit time divided by the number of uninfected hosts at the beginning of the measured time
Density	Another term for mean
Abundance	Another term sometimes used as synonymous with mean
Aggregated	A situation in which most of the parasites occur in a relative minority of hosts
Overdispersed	A term roughly equivalent to aggregated and sometimes used as a synonym
Variance/mean ratio	Quotient of the variance (square of standard deviation of a frequency distribution) divided by the mean; sometimes used as a measure of aggregation
k	The value of a parameter of the negative binomial distribution; usually *k* must be calculated to describe an aggregated parasite population by use of mathematical models

(1) Prevalence:

The percent or decimal fraction of a host population that is actually infected. In practice, a sample of a host population is examined; the number infected is then divided by the number examined. Prevalence can also be used to estimate the probability that a host will be infected, but keep in mind that such probabilities can change fairly quickly with variable transmission conditions.

Example: In a sample of 20 fathead minnows, 10 are infected with *Gyrodactylus hoffmani*. The prevalence of *G. hoffmani* in this sample is 0.50 or 50%.

Graphic representation: Plot prevalence on Y-axis, categories of hosts (sex, collection site, season, etc.) on the X-axis. You end up with a bar graph.

(2) Elaborations of prevalence data:

Prevalence can easily be calculated for any subset of the host population, e.g. demographic subclasses, size groups, etc. Progressive changes in prevalence with progressively changing host population characteristics suggests a biological process at work to alter the probability of infection as hosts move from one category to another (e.g. get older). Similarly, prevalence differences between demographic groups, e.g. sexes, suggest biological processes at work to produce a difference in the probability of infection between the two groups.

Statistical tools: **Contingency tables** are the commonly used tools for testing whether prevalence is different in two different populations. Contingency tables actually test whether prevalence is independent of category analyzed; the results are expressed as a chi-squared value. 2 x 2 tables are probably the most meaningful biologically, but other dimensions may be used, depending on the questions being addressed.

(3) Numbers of parasites per host:

The current commonly accepted term for the number of parasites of a single species in or on an individual host animal is **abundance**; abundance can take the value of zero. The average number of parasites/host in a sample is technically called the **mean abundance**, but I prefer, and will use in class, the conventional term **mean** to refer to this average. The term **intensity** refers to the number of parasites, of a single parasite species in an *infected* host individual; intensity cannot take the value of zero. The average number of parasites per **infected** host is **mean intensity**.

Examples:

Fish #1 has 7 worms in it; the intensity (abundance) is 7.

A sample of 25 fish has a total of 153 worms; 153/25 is the mean abundance.

If only 20 out of the 25 fish actually have worms, then the prevalence is 0.80, or 80%.

If only 20 out of the 25 fish actually have worms, then the mean intensity is 153/20 = 7.65; cf. with mean abundance of 153/25 = 6.12. (Note that you can divide mean abundance by the prevalence and get mean intensity.)

Graphic representation: Bar and line graphs, ideally with error bars (standard deviation, standard error, or variance), with mean abundance or mean intensity on the Y axis and classes or categories of hosts on the X axis. Be sure that your host categories, however, have some biological meaning, e.g. sequential collections at a single site. Abundance

and intensity can also be represented by line, again, depending on the meaning of your sampling categories.

Statistical tools: The commonly used statistical tool for determining whether means of two samples are equal is the **t-test. ANOVA** is also used to test the hypothesis that means of several groups are equal. Technically, both ANOVA and t-tests require that the observations be distributed normally, which few parasitological phenomena are. However, t-test and ANOVA can be done on **ranked observations** and the results are usually technically defensible. Your software has both t-tests (Summary Statistics) and ANOVA. It's not at all clear that use of t-tests and ANOVA to test hypotheses regarding mean intensities are justified biologically. Your statistical software also has programs for performing rank sorting operations, and rank-sorted data sets can then be analyzed using t-tests, ANOVA, or non-parametric methods.

Terminology notes: You will find the term **infrapopulation** in the literature. An infrapopulation is all the parasites "of a species in an individual host at a particular time" (Bush et al., 1997). Intensity is the number of parasite individuals in each host's infrapopulation at the time you sampled the host population. A **component population** includes all the parasites of a particular life cycle stage at a single place and time, and a **suprapopulation** includes all life cycle stages at a single place and time. An infrapopulation of *Gyrodactylus hoffmani* would be all the adult worms on one fathead minnow on July 28, 2000, in the South Platte River at Roscoe. A component population of *Gyrodactylus hoffmani* would be all of the adult worms on all fathead minnows and on any other host species that might be infected on July 28, 2000, in the South Platte River at Roscoe. A suprapopulation would include the component population and all the *Gyrodactylus hoffmani* eggs and *Gyrodactylus hoffmani* ciliated larvae loose in the South Platte River at Roscoe.

(4) Distribution of parasite populations:

A distribution in this context is a **frequency distribution** obtained by plotting the numbers of host individuals against parasite/host classes. The raw data you will need consists of counts of the number of parasites, of a particular species, in each host individual. Raw data tend to look something like this:

Parasite/host classes	**# hosts**
0	25
1	7
2	2
3	0
4	1
--	--
--	--
13	1

In the above example, you collected 36 hosts, 25 of them were uninfected, 7 had one worm, 2 had two worms, etc. This set of numbers is a **frequency distribution**.

Graphic representation: If you plot the number of hosts on the Y-axis, and the parasite/host classes on the X-axis, you will obtain a very familiar (at least to parasitologists) bar graph. The graph in this example reveals a common property of parasite populations, namely aggregation; that is, two hosts, one with 4 parasites and the other with 13 parasites, contain 61% of all the parasites, while the rest of the 34 hosts contain 39%. 6% of the hosts have 61% of the parasites; we say this parasite population is aggregated.

Throughout the summer session we will use this kind of population distribution summary over and over again, "fitting" it to a theoretical curve by means of a computer program.

Statistical tools: Frequency distributions are often analyzed using curve fitting programs. Yours is called **CURVFIT.** These programs require that you enter both your x-axis values and your frequencies (y-axis values) into **data files**, name these files, then call those files up for analysis. The results include a list of theoretical frequencies and a chi-square value that tells you how well the particular curve fits your observations. The parameter values such as mean and variance describe your parasite populations.

(5) Species density distribution:

The species density of a parasite community is the mean of a frequency distribution obtained by plotting the number of host individuals vs. the parasite species/host classes. Whereas in the above example we were considering a single species of parasite, here we may be considering several different species, regardless of how many individual parasites of each species are present.

A typical set of species density data might look like this:

Parasite species/host	Number of hosts
0	10
1	15
2	7
3	3
4	1
5	1

The mean of this distribution, which is the species density of this community of five parasite species, is 1.27. This number has value mainly as a comparison with (1) related host species in the same environment, and (2) the same host species in a different environment.

Graphic representation: The independent variable is parasite species/host classes (0 through 5 in this case) and the dependent variable is number of hosts/class. The plot is a bar graph.

Statistical tools: Species density distributions are typically normally distributed, thus species densities, for example of parasites on related hosts in a single habitat, or the same host species in different habitats, can be compared using t-tests or ANOVA.

(6) Species diversity index:

Our computer programs use the Shannon diversity index known as H'. We calculate diversity for parasite communities in various host species, as well as the same host species in various habitats. The formula for this index is

$$H' = -\sum_{i=1}^{n} p(i)\ln p(i)$$

where H' is the index value, p(i) is the proportion of the ith species in the total collection, and ln p(i) is the natural log of that proportion.

Statistical analysis: You need at least two samples of diversity indices in order to perform a statistical analysis, i.e. a comparison. (A sample consists of several values taken from a presumably homogeneous host population.) H' values are rarely normally distributed, so you may have to compare the diversities using ranked H' values in t-tests or ANOVA.

(7) Niche breadth and overlap:

With some host/parasite combinations, it is appropriate to analyze the relationships of the various parasites not only with the host, but also with respect to one another. This analysis can be done using **niche breadth** and **niche overlap** calculations. In order to use these tools, the parasite must present to the host some attribute that you can measure linearly, e.g. intestinal length, age, etc. This attribute is then considered a "resource" and you calculate the breadth of your parasites' niches on that resource. The niche breadth formula we use is

$$B = 1/\sum_{i=1}^{S} [p(i)]^2 * S$$

where p(i) is the proportion of the parasite species found in the ith unit of the resource and S is the total number of units. You can use this formula to express the structure of an intestinal parasite's niche, for example, by dividing the intestine into ten arbitrary but equal segments, and counting the number of parasites in each, the applying the formula.

Niche overlap is calculated according to the formula

$$\alpha_{ij} = \sum_{n} p(i,h) * p(j,h) * B_i$$

h=1

where α_{ij} is the overlap of *species i* over *species j*. It should be obvious from the formula that you need to calculate reciprocal overlaps.

Example: Ectocommensal peritrich ciliates of the genera *Lagenophrys* and *Rhabdostyla* are found on antennae of crustaceans such as *Hyalella axteca* (Amphipoda). If there are 14 segments on a individual amphipod's second antenna, and the two genera are numerically distributed as follows:

Seg #:	1	2	3	4	5	6	7	8	9	10	11	12	13	14
# *Lageno-phrys*:	4	2	3	1	0	0	0	0	0	0	0	0	0	0
# *Rhabdo-styla*:	12	11	8	9	7	8	5	6	3	4	2	0	0	0

then, on the resource known as antenna length, the niche breadth of *Lagenophrys* is 0.238; the niche breadth of *Rhabdostyla* is 0.655; and the overlap of *Lagenophrys* on *Rhabostyla*'s niche is 0.033.

Graphic representation: One easy way to illustrate distribution on a resource is to make the resource the independent variable then produce a bar graph, roughly a frequency distribution, of each of the species.

Statistical analysis**:** We do not normally do statistical comparisons of niche breadth and overlap values, but if you had a large sample of them, they could be done using t-tests and ANOVA on either raw or ranked observations, depending on the underlying distribution.

(8) Elaborations of data:

In this context, the term "elaborate" means to use the analytical tools to subgroups within the host sample. E.g., we can answer questions such as do females support a more diverse parasite fauna than males? and does *Peromyscus maniculatus* have a richer community of parasites in the hills than in the prairies? using concepts and statistical tools described above. Keep in mind that the number analysis is just the technology required to help you answer a question in a formal scientific way. The real biology behind this work lies in the skill and insight with which you ask the questions and devise the means to gather your data.

14. The Field Parasitology statistical software

One of the things I would like to accomplish this summer is to get each of you to regularly use the computer, and the statistical and quantitative tools described in the previous chapter, in your routine work. You will have plenty of help in trying to accomplish this feat, and I usually have an enormous amount of patience with students who are unfamiliar with either statistics or computers. I encourage you to help one another, to work together in order to learn how to use the software provided, to learn how to express your results verbally, and to increase your statistical sophistication daily. We will use the programs regularly in lab.

My only suggestion is to decide right at the beginning that you are not going to be intimidated by a machine, especially one that depends entirely on your input. If you are very familiar with computers, some of the programs and instructions will seem embarrassingly elementary. If you are afraid of math, statistics, and computers, these same instructions may seem as if they have been written in Martian. The truth is probably somewhere in between.

I have only one truly absolute requirement: THE HARDWARE MUST BE TREATED WITH THE UTMOST RESPECT. THAT IS, THE DISC DRIVES MUST BE USED GENTLY, THE PRINTERS KEPT FREE OF MAJOR DIRT, INSECTS, ETC. There is nothing more effective than a computer breakdown to make this course turn into a great deal of labor in a hurry. Thanks in advance for your cooperation.

I suggest you become familiar with the statistical package entitled FieldStat available on the IBM compatibles. In general, FieldStat is user friendly and easy to learn. I have found that the easiest way to learn to use this package is to use it. Although that advice sounds a little bit circular, the package has built-in instructions that come up on the screen. If you follow the instructions, be patient with yourself and let yourself make a few mistakes at the beginning, then the learning proceeds very quickly. We have Richard E. Clopton to thank for this software. It is among the most powerful and easy to use of any student level statistical programs. Many of the CPBS computers also have SysStat, a commercial program that is powerful but may not be quite so easy to use as FieldStat.

A quick reference guide to DOS commands and command line prompts:

From the **Start** button on your computer, choose **Programs**, then **MS-DOS Prompt**. Or, from the **Start** button, choose **Run**, and type in **command** (enter). You should get a black screen with white characters reading **C:\WINDOWS>** or **C:\DOCUME~1\CEDARPOINT** or something like that. From there, type **cd c:** (enter) and you should get a DOS prompt that looks like **c:\>**. At this point, you are ready to use DOS. The commands let you change directories (= folders), see what is in the various directories (= folders), and execute commands. <u>In DOS, you must always follow your command with an (enter); i.e. press the enter button.</u> There is a table of commands on the next page; the table assumes you press (enter) after typing a command.

Commands	What they do	Example	Result
a:	changes to the floppy drive a		You get the a:\> prompt
ALT	activates the menu bar when you're in the DOS editor		
c:	changes to the hard drive c when you're in the a drive.		You get the c:\> prompt
cd	change directories (folders)	cd fs	You're now in the c:\fs directory
cd c:\	gets you back to the c:\> prompt		
copy	copies files (you have to name them, and designate a destination)		
copy myfile.dat a:\	copies a file named myfile.dat onto the disc in the floppy drive a.		
del	deletes a file (you have to name it).		
del myfile.dat	deletes the file named myfile.dat.		
dir	show you what files are in a directory		
dir c:\fs	shows you what files are in the directory (folder) named FS		
dir c:\fs/w	displays the files across the screen (wide) instead of as a list.		
edit	opens up the DOS editor		
\	the path slash	cd c:\biodiver\data	You're now in the folder (directory) called c:\biodiver\data
*	the "wild card" symbol.	*.csv refers to all files, named anything, but with a csv extension.	

Prompts	What they mean
c:\>	You're in the main c drive directory
c:\fs>	You're in the directory (= folder) named FS

Advice:

(1) No matter what program you're using, limit your file names to 8 characters plus a three character extension. Example: ourfile01.csv

(2) Take a few minutes to read this page and envision doing something simple, such as running a program from DOS.

(3) Any programs that we run from DOS in this lab will have a *.EXE extension.

Spreadsheets:

The CPBS computers all have spreadsheets on them (e.g. Excel). These spreadsheets typically have two characteristics that may be very useful to you:

(1) They have built-in statistical functions that can be used to analyze data; and

(2) They allow data to be saved in files that can then be used in other applications, e.g. graphics programs or FieldStat.

I strongly suggest you become familiar with spreadsheet uses; feel free to ask for individual help in accomplishing this task.

Statistical analysis flow chart/key (R. E. Clopton)

Note: Names in bold caps are names of programs (tools) available in Field Parasitology software packages.

I. Looking for, or trying to test a hypothesis that deals with . . .

A. A relationship?

1. Between independent and dependent data sets?

a. Suspect a straight line relationship?

(1) Yes? Use **LINEAR REGRESSION**

(2) No? Use **CURVFIT**, although the underlying math is not very satisfying

b. Suspect an all or none, or +/- phenomenon?

Use **CONTINGENCY TABLE**

2. Between two co-varying properties?

Use **LINEAR REGRESSION**

B. Differences?

1. Between means and variances of two groups?

a. Normally distributed variables? Use **SUMMARY STATISTICS**

b. Non-normally distributed variables? Use **RANK SORT** to transform data into ranked variates then use **SUMMARY STATISTICS**

2. Between means and variances of more than two groups?

a. Normally distributed variables? Use **ANOVA**

b. Non-normally distributed variables? Use **RANK SORT** to transform data into ranked variates then use **ANOVA**

3. Between fractions of host populations infected?

Use **CONTINGENCY TABLE**

4. Between niche breadth and overlaps?

   Use **NICHE BREADTH**

C. Models?

1. To describe populations of parasites?

   Use **CURVFIT**

2. To describe assemblages of parasite species?

   a. Their species density? Use **CURVFIT, DENSDIST**.

   b. Their species diversity? Use a frequency distribution of diversity indices (see **DIVINDEX)**

II. Preparing for?

A. Use of **CURVFIT, SUMMARY STATISTICS, ANOVA, LINEAR REGRESSION**?

Start by entering data in a file using **DATA EDITOR** or using one of the spreadsheet programs to make your data file (*.csv or comma delimited files, in Excel; *.prn files in Lotus.)

B. Use of **DIVINDEX, NICHE BREADTH, CONTINGENCY TABLE**?

Start by running the program and enter data as asked for it.

III. Trying to interpret?

A. Differences between means, **SUMMARY STATISTICS** or **ANOVA** results?

1. Use Student's t tables for **SUMMARY STATISTICS** results.

2. Use F tables for **ANOVA** results.

3. Decide that the differences, or lack of real differences, means in a biological sense.

B. Results of **LINEAR REGRESSION** analysis?

1. Use proportion of variance attributable to regression as an indication of the amount of change in the dependent variable due to changes in independent variable.

2. Use F tables to determine whether the change is a linear one.

3. Use slope and Y-intercept to draw the line that graphically shows the relationship between independent and dependent variables.

4. Decide what the relationship, or lack thereof, suggests about the biology of the system you are studying.

C. Results of **DIVINDEX** or diversity index calculations?

The diversity index values don't mean much by themselves; they need to have some comparative diversity index values, for example, from the same host/parasite system in different environments, or from different host/parasite systems in the same environment. You will need to consider the species of hosts and parasites involved, the nature of their transmission mechanisms, etc., in order to decide what kind of meaning diversity index calculations have.

D. Results of **CURVFIT** calculations?

CURVFIT gives you a chi-squared value and degrees of freedom for three or four theoretical frequency distributions fit to your data, based primarily on your number of observations and observed mean and variance. The lowest chi-squared value corresponds to the curve of best fit. The curve of best fit is the best theoretical description of your sample (usually a population of parasites). These analyses yield the most information when they are comparative, as in the case of diversity indices.

E. Results of **CONTINGENCY TABLE** calculations?

CONTINGENCY TABLE gives you a chi-squared value and degrees of freedom. Use the table to determine whether categories, e.g. infected or non-infected, are independent of other categories, e.g. male and female, in the same set of hosts. You must supply the biological part of the interpretation.

FINAL NOTE: All of these programs are simply tools for testing null hypotheses, i.e. hypotheses of no difference between groups or between observations and a model. The tools work most satisfactorily when the hypotheses are phrased to actually test some biological principle, some assertion about the way organisms exist in nature, and when the data gathered actually let you test the hypotheses both biologically and statistically. These tools also typically carry assumptions about normality of underlying distributions, equality of variances, etc., so be aware that my teaching strategy involves getting you first to use these regularly, then second to increase the sophistication with which you use them.

NOTE RE FP2000: The FieldStat package has also been upgraded to one called FS2. However, this upgrade may not be completely finished yet, and in particular there may be undetected bugs yet to be corrected.

15. Individual Research Projects

The educational objective of a research project in a course is to teach you something about the trials, tribulations, joys, etc. of (1) deciding what you would like to study, (2) gathering the resources necessary to do the project, (3) making the observations necessary to test a hypothesis, produce a discovery, etc., (4) putting the experience into words in writing, and (5) telling other scientists (the class) the results of your investigations. Obviously, the actual results, the questions addressed and answered, are all of secondary importance when the research is done as a class requirement. Thus it is not expected that you will win a Nobel Prize for your project. It is expected that you will give it your best try. Such small research projects are one of the most effective devices teachers have for teaching transferable skills.

The most educational projects, and in many ways most satisfying to the student, are ones which employ small creatures, small systems, that can be collected in large numbers and require no collecting permit. Survey projects are ones which simply ask: who is parasitized by whom? Logical extensions of surveys include host specificity observations, demographic observations, and testing of predictions about parasite populations and assemblages in various hosts, or in diverse environments occupied by a host species.

The most sophisticated projects are built around a non-trivial hypothesis or prediction and involve design of the study in order to gather the observations which provide a true test of the hypothesis or prediction. While this general intellectual approach is the essence of scientific experimentation, in practice, it is sometimes possible to phrase your predictions in a way so that they can be tested without actually maintaining and manipulating biological materials, e.g. through specifically designed surveys. Experimentation has not been an overly popular type of approach to CPBS parasitology projects, mainly, I suspect, because we don't teach experimentation in Lincoln and the logistics of doing experiments in five week sessions are sometimes overwhelming, especially when wild animals are involved. However, some of our most interesting projects have been experiments; the key to success will be in choosing your materials and the appropriate questions.

Don’t worry too much if you don’t “solve your problem.” Chances are, you will uncover other problems perhaps more interesting than the one you investigated. And that is the way much of science has operated for a long long time. I will point out many potential projects, especially in the first week. In addition, I will mention some of the advantages and pitfalls of these projects. In particular, when there are small projects that can be done in a way that illustrates a parasitological principle, or will answer a profound parasitological question, then I will encourage someone to work on that project.

Be sure to budget time to work on your project regularly. All scientists do research on a regular basis, and a part of their day to day activities, and your own research experience will go most smoothly if you do the same. You will very likely need some help in finding equipment, glassware, space, and perhaps chemicals. Be sure to ask for this help early, and to ask for advice and help on other aspect of your project at any time.

Please be very respectful of other people's projects, especially when they need to have things set up in the lab. You will discover very quickly that research requires a considerable investment of time and energy, so that your materials become quite valuable.

Finally, perhaps the best advice is: don't feel a need to impress other people, especially me, with the sophistication and ambition of your project. Remember, this is an educational experience; I'd rather you work hard and do a small project well than fumble around with an elegant and ambitious one that goes nowhere.

Projects work best if they are done with a partner. Pick out someone you can work with and get along with. Generally speaking, project partners are of most help to one another when they are chosen for intellectual, rather than social, reasons. Projects are also most satisfying if they are not the same ones done by previous students; originality is always more fun than repeating someone else's work.

Plan to have your project selected and started by the end of the first week.

Writing your paper

It is a class policy that in Field Parasitology project reports will be written in the form of a manuscript to be submitted to the Journal of Parasitology. Before you sit down to write, consult the Journal's instructions to authors and follow the indicated format. The instructions are given in the Appendix to this manual. You will notice some very real differences between the journal manuscript format and the one you might be familiar with from English classes. If you have questions about the format, please ask well in advance of the last week.

In practice, most scientists write the results section of their papers first. In fact, the easiest way to write a scientific paper is to get all your tables and figures prepared first, in final form, in sequence, then write the results section. The second section to write is the materials and methods. Write the discussion third. Then put together the literature cited, making sure not to include anything you have not referred to in the text, or vice versa. Write the introduction then the abstract. The abstract should actually reveal what you did and what you discovered. If you proceed in this sequence, your paper will read as if you actually knew what you were doing from the very beginning.

In this course, the writing of your research paper, based on your project, is a "role playing" exercise. Thus it is important to try to follow the Journal of Parasitology manuscript guidelines as exactly as possible, given the resources at your disposal. The paper should be submitted with a cover letter addressed to the JP editor.

16. Oral Presentations

A general guide for oral presentations is this:

Statement of the problem and reasons for studying the problem	1-2 minutes
Methods	2 minutes
Results	4 minutes
Meaning/interpretation	2 minutes
Total time	10 minutes

Transparencies for use on the overhead projector can be made from acetate provided, using a marking pen of some kind. If you use the photocopy machine for making transparencies, be sure to use the correct kind of acetate or you will melt the thing inside the machine. Be simple but artistic with your graphic design; long tables with numbers are routinely boring and non-instructional. Most research teams divide up the presentation between team members. Don't be too upset if your talk runs 15 minutes, but the audience gets very impatient if all the papers are too long.

Remember that oral presentation is part of the educational process in which we act like professional scientists. The fact that the presentation is made on results from a study *you* chose to pursue is one of the most interesting and exciting aspects of the papers, and for that reason, I usually try to ask for an abstract and make up an official-looking program.

Hints on how to maximize your grade and add authenticity to your educational experience:

(1) Speak loudly.

(2) Use elegant and grammatically correct spoken English.

(3) Ask for questions at the end.

(4) Know your material.

(5) Dress reasonably well (for CPBS!); don't wear a cap.

(6) Practice using the pointer, overhead, video, or other AV aids.

17. Safety Issues

Safety is a matter of continuous concern at CPBS, and the Field Parasitology course is certainly no exception. You will receive a considerable amount of safety information during the opening station meeting. The following advice, information, and suggestions are intended to supplement the introductory safety material received on Sunday, the first day of the session.

(1) It is a state law that anyone working in a laboratory with chemicals must be wearing protective eyewear. Thus when you're over at the specimen table, you should have on your protective glasses.

(2) Electricity is always a concern with courses that use a lot of aquatic materials, and Field Parasitology uses a LOT of water. Please use extra caution when plugging in your microscopes and lamps, and unplugging them. Let us know if there are frayed cords.

(3) Field Parasitology also uses a lot of glassware, especially petri dishes and pipets, which are easily broken. Thus I strongly recommend always wearing shoes in the lab (and in fact everywhere at Cedar Point), and being particularly cautious when reaching into a tub of dirty dishes.

(4) Rubber gloves are available for those of you who wish to use them. I've never used them and I've never gotten sick from anything we've done with parasites, hosts, or mud/water in class at Cedar Point. However, certain animal species, e.g. skunks, opossums, raccoons, small rodents, and canids, are potential carriers of organisms infective to humans. For this reason, we don't use small rodents in class any more (rabbits are not rodents). I would always use rubber gloves when messing with any mammal, and I no longer touch wild canids.

(5) The major chemicals we use are:

FAA (or AFA): formaldehyde, ethyl alcohol, and acetic acid (fixative) – caustic and irritating to the eyes. You should not breath formaldehyde vapors; formaldehyde is a possible carcinogen.

EtOH: Ethyl alcohol – solvent, dehydrating agent, irritating to open cuts and eyes, flammable at higher concentrations.

Xylene: organic solvent and clearing agent, irritating to open cuts and eyes, fairly flammable; you should not breath xylene vapors.

Carmine stain: carmine itself is not very dangerous, but it's mixed in 50% glacial acetic acid, which can burn you.

Methanol: methyl alcohol, used for fixing blood and tissue smears, Indy Car fuel, highly flammable and burns with an invisible flame, thus can be quite dangerous when on fire.

Giemsa stain: the powdered stain is not very toxic; made up in water, glycerin, and methanol; used for staining blood and tissue smears. The prepared stain will make you (and your clothes) blue for a while.

Hydrochloric acid (HCl): used to make destain (10% HCl in 70% EtOH); extremely caustic; TA usually makes destain; HCl is always used outside.

Glacial acetic acid: used to fix and straighten nematodes; extremely caustic and flammable; used outside.

Sodium hydroxide (NaOH): used to bleach arthropods (10% solution in water); extremely caustic in dry form and high concentrations.

(6) Dissecting instruments are always a problem in parasitology labs because we are always using them on strange organisms and tissues, and we are always sharpening them. Please be careful with your eyes and fingers. I would avoid using razor blades and scalpels.

(7) The CPBS administration will talk about general safety issues, e.g. traffic, insects, sunburn, poison ivy, barbed wire fences, sticks in eyes, shop tools, recreational use of surrounding land, etc., at the opening Sunday orientation meeting. Please pay close attention to that presentation.

(8) On field trips, pay particular attention to water safety, and be sure to tell us if you do not swim, or are afraid of being in water that may be over knee deep. We also often go collecting in places that are quite muddy, and I would never put any of that material into your mouth.

(9) We do pick up road kills when it is safe to do so. Please make sure that any road kill you pick up is actually dead, and if it is not very very fresh, don't pick it up. I would not pick up skunks, coyotes, foxes, or small rodents.

(10) If you have any safety questions or concerns, please feel free to ask me, the TA, or the CPBS administration.

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FIELD PARASITOLOGY COLLECTION RECORD

Name_____ Year_____

Cat #	Date	Locality	Latitude	Longitude	Host	Parasite	Inf. site

Chi-Squared Table (single-tailed)

Single-tailed chi squared distribution percentiles										
df	**0.5**	**1**	**2.5**	**5.0**	**10.0**	**90.0**	**95.0**	**97.5**	**99.0**	**99.5**
1	3.9E-05	2E-04	1E-03	0.004	0.02	2.71	3.84	5.02	6.63	7.88
2	0.01	0.02	0.05	0.10	0.21	4.61	5.99	7.38	9.21	10.60
3	0.07	0.12	0.22	0.35	0.58	6.25	7.81	9.35	11.34	12.84
4	0.21	0.30	0.48	0.71	1.06	7.78	9.49	11.14	13.28	14.86
5	0.41	0.55	0.93	1.15	1.61	9.24	11.07	12.83	15.09	16.75
6	0.68	0.87	1.24	1.64	2.20	10.64	12.59	14.45	16.81	18.55
7	0.99	1.24	1.69	2.17	2.83	12.02	14.07	16.01	18.48	20.28
8	1.34	1.65	2.18	2.73	3.49	13.36	15.51	17.53	20.09	21.06
9	1.73	2.09	2.70	3.33	4.17	14.68	16.92	19.02	21.67	23.59
10	2.16	2.56	3.25	3.94	4.87	15.99	18.31	20.48	23.21	25.19
11	2.60	3.05	3.82	4.57	5.58	17.28	19.68	21.92	24.73	26.76
12	3.07	3.57	4.40	5.23	6.30	18.55	21.03	23.34	26.22	28.30
13	3.57	4.11	5.01	5.89	7.04	19.81	22.36	24.74	29.69	29.82
14	4.07	4.66	5.63	6.57	7.79	21.06	23.68	23.12	29.14	31.32
15	4.60	5.23	6.26	7.26	8.55	22.31	25.00	27.49	30.58	32.80
16	5.14	5.81	6.91	7.96	9.31	23.54	26.30	28.85	32.00	34.27
18	6.26	7.01	8.23	9.39	10.86	25.99	28.87	31.53	34.81	37.18
20	7.43	8.26	9.59	10.85	12.44	28.41	31.41	34.17	37.57	40.00
24	9.89	10.86	12.40	13.85	15.66	33.20	36.42	39.36	42.98	45.56
30	13.79	14.96	16.79	18.49	20.60	40.26	43.77	46.98	50.89	53.67
40	20.71	22.16	24.43	26.51	29.05	51.81	55.76	59.34	63.69	66.77
60	35.53	37.48	40.48	43.19	46.46	74.40	79.08	83.30	88.38	91.95
120	83.85	86.92	91.58	95.90	100.62	140.23	146.57	152.31	158.95	163.64

Percentiles of the *t*-distribution

Single-tailed *t* distribution percentiles								
df	**0.60**	**0.70**	**0.80**	**0.90**	**0.95**	**0.975**	**0.990**	**0.995**
1	0.325	0.727	1.376	3.078	6.314	12.706	31.821	63.657
2	0.289	0.617	1.061	1.886	2.291	4.303	6.695	9.925
3	0.277	0.584	0.978	1.638	2.353	3.182	4.541	5.841
4	0.271	0.569	0.941	1.533	2.132	2.776	3.747	4.604
5	0.267	0.559	0.921	1.476	2.015	2.571	3.365	4.032
6	0.265	0.553	0.906	1.441	1.943	2.447	3.143	3.707
7	0.263	0.549	0.896	1.415	1.895	2.365	2.998	3.449
8	0.262	0.546	0.889	1.397	1.861	2.306	2.896	3.355
9	0.261	0.543	0.883	1.383	1.833	2.262	2.821	3.251
10	0.261	0.542	0.879	1.372	1.812	2.228	2.764	3.169
11	0.261	0.541	0.876	1.363	1.796	2.201	2.718	3.106
12	0.259	0.539	0.873	1.356	1.782	2.179	2.861	3.055
13	0.259	0.538	0.871	1.351	1.771	2.161	2.651	3.012
14	0.258	0.537	0.868	1.345	1.761	2.145	2.624	2.977
15	0.258	0.536	0.866	1.341	1.753	2.131	2.602	2.947
16	0.258	0.535	0.865	1.337	1.746	2.121	2.583	2.921
17	0.257	0.534	0.863	1.333	1.741	2.111	2.567	2.898
18	0.257	0.534	0.862	1.331	1.734	2.101	2.552	2.878
19	0.257	0.533	0.861	1.328	1.729	2.093	2.539	2.861
20	0.257	0.533	0.861	1.325	1.725	2.086	2.528	2.845
21	0.257	0.532	0.859	1.323	1.721	2.081	2.518	2.831
22	0.256	0.532	0.858	1.321	1.717	2.074	2.508	2.819
23	0.256	0.532	0.858	1.319	1.714	2.069	2.501	2.807
24	0.256	0.531	0.857	1.318	1.711	2.064	2.492	2.797
25	0.256	0.531	0.856	1.316	1.708	2.061	2.485	2.787
26	0.256	0.531	0.856	1.315	1.706	2.056	2.479	2.779
27	0.256	0.531	0.855	1.314	1.703	2.052	2.473	2.771
28	0.256	0.531	0.855	1.313	1.701	2.048	2.467	2.763
29	0.256	0.531	0.854	1.309	1.699	2.045	2.462	2.756
30	0.256	0.531	0.854	1.305	1.697	2.042	2.457	2.751
40	0.255	0.529	0.851	1.303	1.684	2.021	2.423	2.704
60	0.254	0.527	0.848	1.296	1.671	2.001	2.391	2.661
120	0.254	0.526	0.845	1.289	1.658	1.981	2.358	2.617
inf	0.253	0.524	0.842	1.282	1.645	1.961	2.326	2.576

F tables (alpha = 0.05)

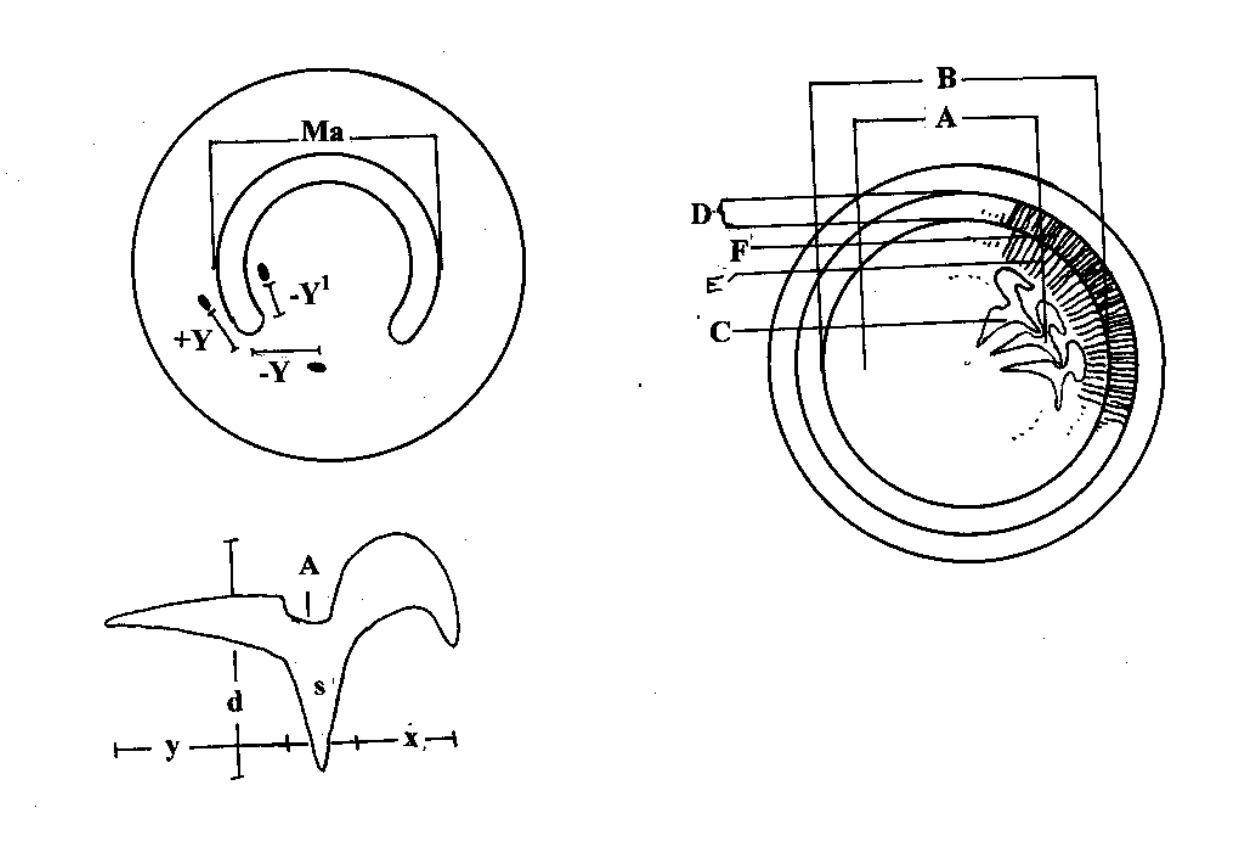
F distribution @ alpha = 0.05

df(d	df (numerator)																				
	1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	inf
1	161	200	216	225	230	234	237	239	241	242	244	246	248	249	250	251	252	253	254
2	18.5	19.0	19.2	19.3	19.3	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.4	19.5	19.5	19.5	19.5	19.5	19.5
3	10.1	9.55	9.28	9.12	9.01	8.94	8.80	8.85	8.81	8.79	8.74	8.70	8.66	8.64	8.62	8.59	8.57	8.55	8.53
4	7.71	6.94	6.59	6.39	6.26	6.16	6.09	6.04	6.00	5.96	5.91	5.86	5.80	5.77	5.75	5.72	5.69	5.66	5.63
5	6.61	5.79	5.41	5.19	5.05	4.95	4.88	4.82	4.77	4.74	4.68	4.62	4.56	4.53	4.50	4.46	4.43	4.40	4.37
6	5.99	5.14	4.76	4.53	4.39	4.28	4.21	4.15	4.10	4.06	4.00	3.94	3.87	3.84	3.81	3.77	3.74	3.70	3.67
7	5.59	4.74	4.35	4.12	3.97	3.87	3.79	3.73	3.68	3.64	3.57	3.51	3.44	3.41	3.38	3.34	3.30	3.27	3.23
8	5.32	4.46	4.07	3.84	3.69	3.58	3.50	3.44	3.39	3.35	3.28	3.22	3.15	3.12	3.08	3.04	3.01	2.97	2.93
9	5.12	4.26	3.86	3.63	3.48	3.37	3.29	3.23	3.18	3.14	3.07	3.01	2.94	2.90	2.86	2.83	2.79	2.75	2.71
10	4.96	4.10	3.71	3.48	3.33	3.22	3.14	3.07	3.02	2.98	2.91	2.85	2.77	2.74	2.70	2.66	2.62	2.58	2.54
11	4.84	3.98	3.59	3.36	3.20	3.09	3.01	2.95	2.90	2.85	2.79	2.72	2.65	2.61	2.57	2.53	2.49	2.45	2.40
12	4.75	3.89	3.49	3.26	3.11	3.00	2.91	2.85	2.80	2.75	2.69	2.62	2.54	2.51	2.47	2.43	2.38	2.34	2.30
13	4.67	3.81	3.41	3.18	3.03	2.92	2.83	2.77	2.71	2.67	2.60	2.53	2.46	2.42	2.38	2.34	2.30	2.25	2.21
14	4.60	3.74	3.34	3.11	2.96	2.85	2.76	2.70	2.65	2.60	2.53	2.46	2.39	2.35	2.31	2.27	2.22	2.18	2.13
15	4.54	3.68	3.29	3.06	2.90	2.79	2.71	2.64	2.59	2.54	2.48	2.40	2.33	2.29	2.25	2.20	2.16	2.11	2.07
16	4.49	3.63	3.24	3.01	2.85	2.74	2.66	2.59	2.54	2.49	2.42	2.35	2.28	2.24	2.19	2.15	2.11	2.06	2.01
17	4.45	3.59	3.20	2.96	2.81	2.70	2.61	2.55	2.49	2.45	2.38	2.31	2.23	2.19	2.15	2.10	2.06	2.01	1.96
18	4.41	3.55	3.16	2.93	2.77	2.66	2.58	2.51	2.46	2.41	2.34	2.27	2.19	2.15	2.11	2.06	2.02	1.97	1.92
19	4.38	3.52	3.13	2.90	2.74	2.63	2.54	2.48	2.42	2.38	2.31	2.23	2.16	2.11	2.07	2.03	1.98	1.93	1.88
20	4.35	3.49	3.10	2.87	2.71	2.60	2.51	2.45	2.39	2.35	2.28	2.20	2.12	2.08	2.04	1.99	1.95	1.90	1.84
21	4.32	3.47	3.07	2.84	2.68	2.57	2.49	2.42	2.37	2.32	2.25	2.18	2.10	2.05	2.01	1.96	1.92	1.87	1.81
22	4.30	3.44	3.05	2.82	2.66	2.55	2.46	2.40	2.34	2.30	2.23	2.15	2.07	2.03	1.98	1.94	1.80	1.84	1.78
23	4.28	3.42	3.03	2.80	2.64	2.53	2.44	2.37	2.32	2.27	2.20	2.13	2.05	2.01	1.96	1.91	1.86	1.81	1.76
24	4.26	3.40	3.01	2.78	2.62	2.51	2.42	2.36	2.30	2.25	2.18	2.11	2.03	1.98	1.94	1.89	1.84	1.79	1.73
25	4.24	3.39	2.99	2.76	2.60	2.49	2.40	2.34	2.28	2.24	2.16	2.09	2.01	1.96	1.92	1.87	1.82	1.77	1.71
30	4.17	3.32	2.92	2.69	2.53	2.42	2.33	2.27	2.21	2.16	2.09	2.01	1.93	1.89	1.84	1.79	1.74	1.68	1.62
40	4.08	3.23	2.84	2.61	2.45	2.34	2.25	2.18	2.12	2.08	2.00	1.92	1.84	1.79	1.74	1.69	1.64	1.58	1.51
60	4.00	3.15	2.76	2.53	2.37	2.25	2.17	2.10	2.04	1.99	1.92	1.84	1.75	1.70	1.65	1.59	1.53	1.47	1.39
120	3.92	3.07	2.68	2.45	2.29	2.18	2.09	2.02	1.96	1.91	1.83	1.75	1.66	1.61	1.55	1.50	1.43	1.35	1.25
inf	3.84	3.00	2.60	2.37	2.21	2.10	2.01	1.94	1.88	1.83	1.75	1.67	1.57	1.52	1.46	1.39	1.32	1.22	1.00

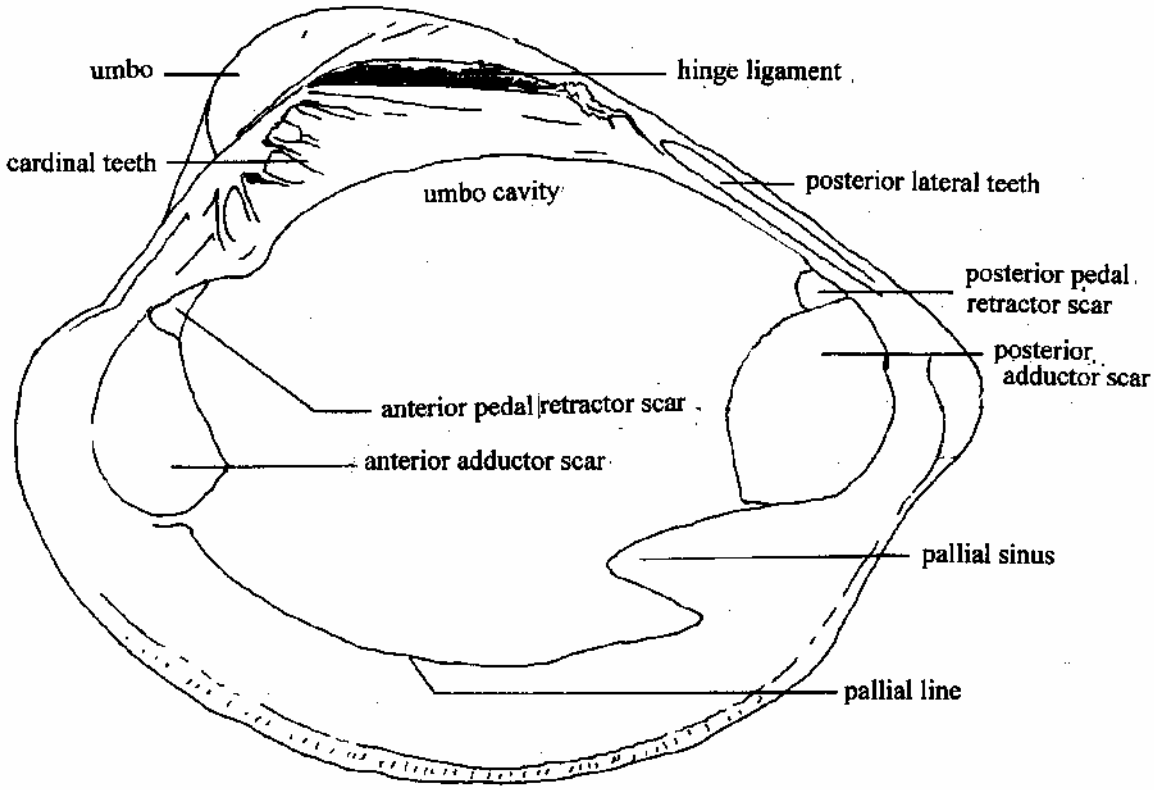
Significant values for correlation coefficients

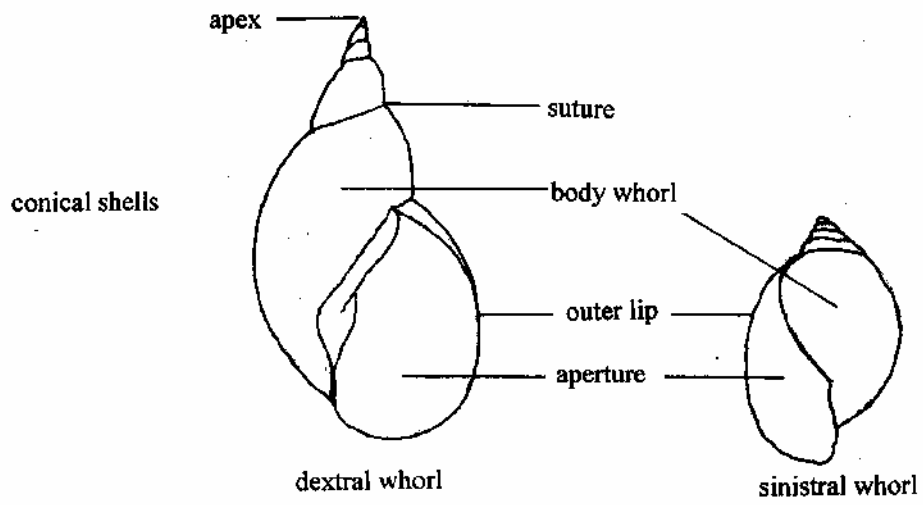
df	α	Nmbr indpent variables 1	2	3	4	df	α	Nmbr indpent variables 1	2	3	4	df	α	Nmbr indpent variables 1	2	3	4
1	0.05	0.997	0.999	0.999	0.999	19	0.05	0.433	0.521	0.575	0.615	80	0.05	0.217	0.269	0.304	0.332
1	0.01		1	1	1	19	0.01	0.549	0.621	0.665	0.698	80	0.01	0.283	0.331	0.362	0.389
2	0.05	0.951	0.975	0.983	0.987	20	0.05	0.423	0.509	0.563	0.604	90	0.05	0.205	0.254	0.288	0.315
2	0.01	0.991	0.995	0.997	0.998	20	0.01	0.537	0.608	0.652	0.685	90	0.01	0.267	0.312	0.343	0.368
3	0.05	0.878	0.93	0.95	0.961	21	0.05	0.413	0.498	0.522	0.592	100	0.05	0.195	0.241	0.274	0.301
3	0.01	0.959	0.976	0.983	0.987	21	0.01	0.526	0.596	0.641	0.674	100	0.01	0.254	0.297	0.327	0.351
4	0.05	0.811	0.881	0.912	0.931	22	0.05	0.404	0.488	0.542	0.582	125	0.05	0.174	0.216	0.246	0.269
4	0.01	0.917	0.949	0.962	0.971	22	0.01	0.515	0.585	0.631	0.663	125	0.01	0.228	0.266	0.294	0.316
5	0.05	0.754	0.836	0.874	0.898	23	0.05	0.396	0.479	0.532	0.572	150	0.05	0.159	0.198	0.225	0.247
5	0.01	0.874	0.917	0.937	0.949	23	0.01	0.505	0.574	0.619	0.652	150	0.01	0.208	0.244	0.271	0.291
6	0.05	0.707	0.795	0.839	0.867	24	0.05	0.388	0.471	0.532	0.562	200	0.05	0.138	0.172	0.196	0.215
6	0.01	0.834	0.886	0.911	0.927	24	0.01	0.496	0.565	0.609	0.642	200	0.01	0.181	0.212	0.234	0.253
7	0.05	0.666	0.758	0.807	0.838	25	0.05	0.381	0.462	0.514	0.553	300	0.05	0.113	0.141	0.161	0.176
7	0.01	0.798	0.855	0.885	0.904	25	0.01	0.487	0.555	0.601	0.633	300	0.01	0.148	0.174	0.192	0.208
8	0.05	0.632	0.726	0.777	0.811	26	0.05	0.374	0.454	0.506	0.546	400	0.05	0.098	0.122	0.139	0.153
8	0.01	0.765	0.827	0.861	0.882	26	0.01	0.478	0.546	0.591	0.624	400	0.01	0.128	0.151	0.167	0.181
9	0.05	0.602	0.697	0.751	0.786	27	0.05	0.367	0.446	0.498	0.536	500	0.05	0.088	0.109	0.124	0.137
9	0.01	0.735	0.801	0.836	0.861	27	0.01	0.471	0.538	0.582	0.615	500	0.01	0.115	0.135	0.151	0.162
10	0.05	0.576	0.671	0.726	0.763	28	0.05	0.361	0.439	0.491	0.529	1000	0.05	0.062	0.077	0.088	0.097
10	0.01	0.708	0.776	0.814	0.84	28	0.01	0.463	0.531	0.573	0.606	1000	0.01	0.081	0.096	0.108	0.115
11	0.05	0.553	0.648	0.703	0.741	29	0.05	0.355	0.432	0.482	0.521						
11	0.01	0.684	0.753	0.793	0.821	29	0.01	0.456	0.522	0.585	0.598						
12	0.05	0.532	0.627	0.683	0.722	30	0.05	0.349	0.426	0.476	0.514						
12	0.01	0.661	0.732	0.773	0.802	30	0.01	0.449	0.514	0.558	0.591						
13	0.05	0.514	0.608	0.664	0.703	35	0.05	0.325	0.397	0.445	0.482						
13	0.01	0.641	0.712	0.755	0.785	35	0.01	0.418	0.481	0.523	0.556						
14	0.05	0.497	0.591	0.646	0.686	40	0.05	0.304	0.373	0.419	0.455						
14	0.01	0.623	0.694	0.737	0.768	40	0.01	0.393	0.454	0.494	0.526						
15	0.05	0.482	0.574	0.631	0.671	45	0.05	0.288	0.353	0.397	0.432						
15	0.01	0.606	0.677	0.721	0.752	45	0.01	0.372	0.431	0.471	0.501						
16	0.05	0.468	0.559	0.615	0.655	50	0.05	0.273	0.336	0.379	0.412						
16	0.01	0.591	0.662	0.706	0.738	50	0.01	0.354	0.411	0.449	0.479						
17	0.05	0.456	0.545	0.601	0.641	60	0.05	0.251	0.308	0.348	0.381						
17	0.01	0.575	0.647	0.691	0.724	60	0.01	0.325	0.377	0.414	0.442						
18	0.05	0.444	0.532	0.587	0.628	70	0.05	0.232	0.286	0.324	0.354						
18	0.01	0.561	0.633	0.678	0.711	70	0.01	0.302	0.351	0.386	0.413						

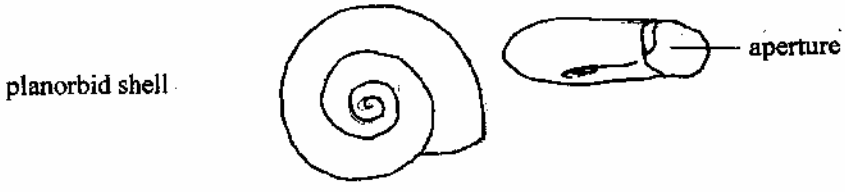
How to measure a *Trichodina* specimen:



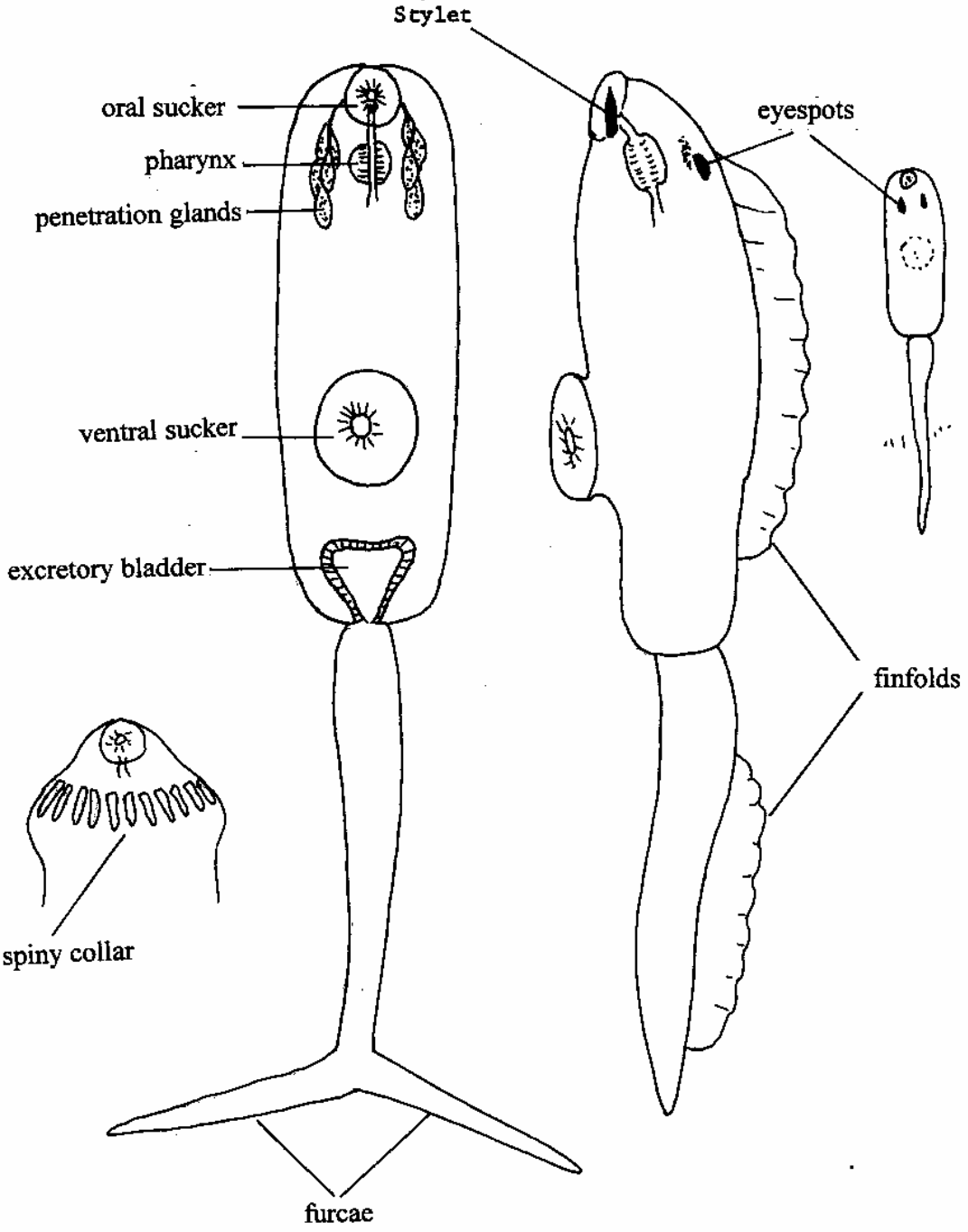
Molluscan anatomy



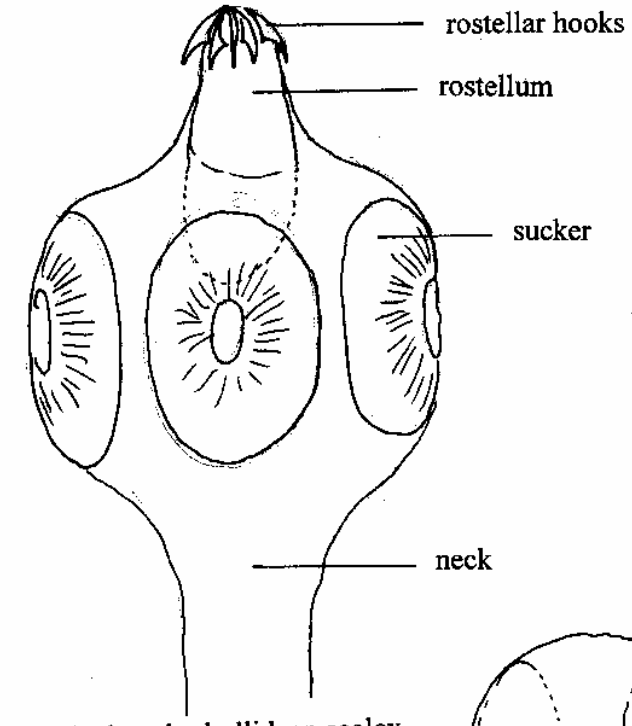




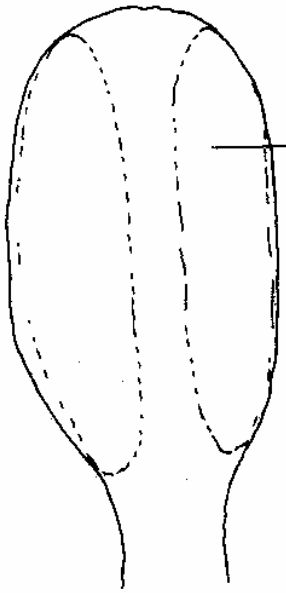
Cercarial anatomy (a composite drawing):



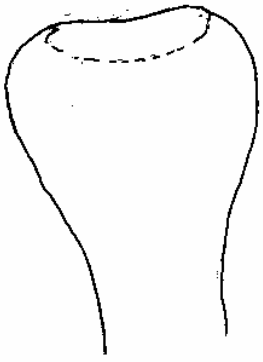
Scolex anatomy:



A typical cyclophyllidean scolex



A typical pseudophyllidean scolex



A typical caryophyllidean scolex